THE GAMETOPHYTE OF *STENOCHLAENA PALUSTRIS* (BURM.) BEDD.

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In view of the difference of opinion about the systematic position of the small genus *Stenochlaena* it is desirable to have some knowledge of the gametophyte stage. We are indebted to Professor R. E. Holttum for the spores of *Stenochlaena palustris* (Burm.) Bedd. which he kindly sent us from Singapore in May 1950.

The spores were planted immediately on various media: distilled water, silica gel with nutrient salts, porous clay crock, and peat. Germination took place in 7-8 days on water, but was slower on silica gel and those cultures were never satisfactory. The cultures on peat grew well and after 19 months are still flourishing. Spores 18 months old gave a low percentage of germination and this required about three weeks. This may have been caused in part by the less favourable conditions of light in November and December, but younger spores of other species were not perceptibly delayed.

Much of the investigation was made on fresh material, but for the sex organs, particularly the archegonium, sections were made from material embedded in paraffin after fixation with various fluids. The best proved to be Manton's chromacetic formalin mixture (Manton, 1950) diluted one-half with water, and formalin-propionic-alcohol (FPA). The latter was preferred as the rapid penetration insured division figures, and it avoids the precipitation which stains so heavily in the neck cells following fixation in the former. Sections for antheridia were cut at 6 μ, and those for archegonia at 6-10 μ. Haidenhein's haematoxylin counterstained with fast green or orange G was preferred, but the combination of safranin-crystal violet-orange G was also used.

The spores of *S. palustris* are bilateral, distinctly yellow, without a perispore but with pronounced tubercles or blunt processes, and average 38×27 μ in size (Fig. 1). The approach of germination is indicated by the change in appearance of the contents which take on a greenish tinge and become more opaque with definite droplets or granules. When the spore coat cracks, a papilla emerges as the primary prothallial cell with chloroplasts soon visible in the tip and fat globules in the basal region. As is characteristic of the higher ferns, the first wall cuts off the first rhizoid; this has few, if any, chloroplasts (Fig. 2, *a-c*). The green cell elongates and a filament is formed by a series of cross walls (Fig. 3, *a-c*). The filament may be 4-7 cells long, seldom longer, before its growth in length is checked by the formation of a papillate hair on the terminal cell (Fig. 3, *b, c*). The formation of such a hair was of regular occurrence in our material. The length of the cells in the filament varies with conditions and they tend to become elongated in weak light or crowded cultures (Fig. 3, *c*).

The broadening of the filament into a plate is brought about by longitudinal divisions in cells behind the terminal cell. The third cell from the tip is usually the most active, but the second, fourth and even those farther back may have longitudinal divisions and contribute to the formation of the plate (Figs. 4, 5); on some gametophytes the basal cell is the only one left undivided (Fig. 6). A wedge-shaped apical cell is formed in a more or less lateral position (Fig. 6), but the growth of the thallus is such that
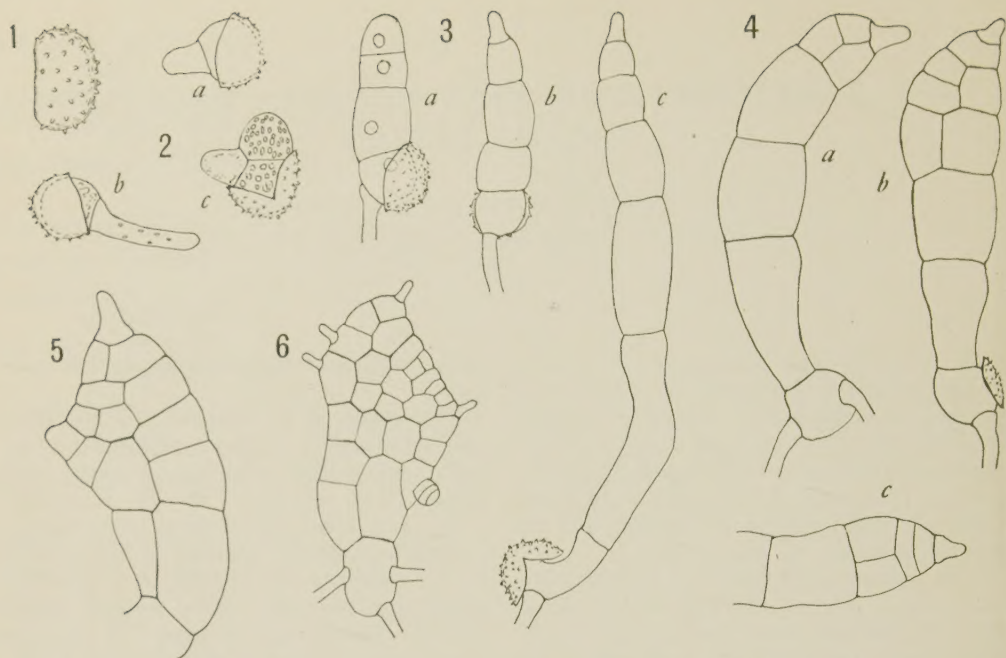
FIGS. 1-6 — Fig. 1, spore. ×350. Fig. 2, germination stages. ×225. Fig. 3, development of filament. Figs. 4, 5, beginning of plate formation. Fig. 6, young gametophyte with apical cell and antheridium.

the cell which was originally terminal is pushed to a lateral position (*t*, Fig. 7), and the apical meristem is seen sooner or later in a notch near the centre of the longitudinal axis of the plate (Figs. 8, 9). The extent of activity of the apical cell was not determined, but it is eventually replaced by a marginal meristematic group (Fig. 8). By this time the gametophyte has become approximately symmetrical with a deep notch and well-developed wings. The developing thallus forms a midrib in the usual way and soon becomes broadly cordate (Fig. 9, thallus 50 days old). The midrib is not heavy in young gametophytes, but if fertilization does not occur and growth continues, a midrib 8-9 cells thick (Fig. 14) may be formed on such a thallus as that in Fig. 10. In old cultures branching of the midrib is found occasionally, and there develop two or three meristematic regions which produce archegonia (Fig. 11).

Young rhizoids are colourless but occasionally contain a few chloroplasts which soon disappear (Fig. 2*b*). The old rhizoids are stout with a heavy wall which is tan, straw-coloured, or more often reddish-brown. The basal cell of the filament bears one rhizoid usually, but may have two or three (Figs. 3, 4, 6). Rhizoids are found on marginal cells of young gametophytes but on those approaching maturity they are formed on the midrib or near it. The growth of rhizoids on old gametophytes is very heavy suggesting tawny wool; every vegetative cell on the ventral side of the midrib apparently bears a rhizoid and the mass of rhizoids completely conceals the sex organs. Septations are not infrequent and the rhizoid may break with a sharp line at the septum (Fig. 12).

Papillate hairs containing chloroplasts develop on the marginal cells of the young gametophyte, and also, somewhat later, on the surface (Figs. 8, 9, 13). The hairs are typically unicellular, but a septate hair is found occasionally (Fig. 13*b*). The hairs retain their chloroplasts for a

long time, but the plastids grow smaller and eventually disappear. In the early stages there is little indication of glandular character (Fig. 13*e*), but later there may be a slight secretion. The hairs on the surface stand erect (Fig. 13*a*, *b*) and in general are longer than those on the margin (Fig. 13*c*, *d*).

Antheridium

Antheridia may appear at the early plate stage of the gametophyte when it is about 20 days old (Fig. 6, 8), but they are not abundant until several weeks later. They appear in considerable num- bers on gametophytes which later bear archegonia, but usually do not continue to form after archegonium production begins (Fig. 9). Antheridia may be found developing again, if for any reason archegonium production is checked; zones of antheridia may then be found inter- polated between zones of archegonia on old gametophytes. Antheridia are borne chiefly on the ventral surface, sometimes on the dorsal, and less frequently on the margins, except in the case of regenerated branches. Ameristic male prothalli were not of common occurrence in our material, but the elongated slender prothalli found in crowded cultures sometimes bore
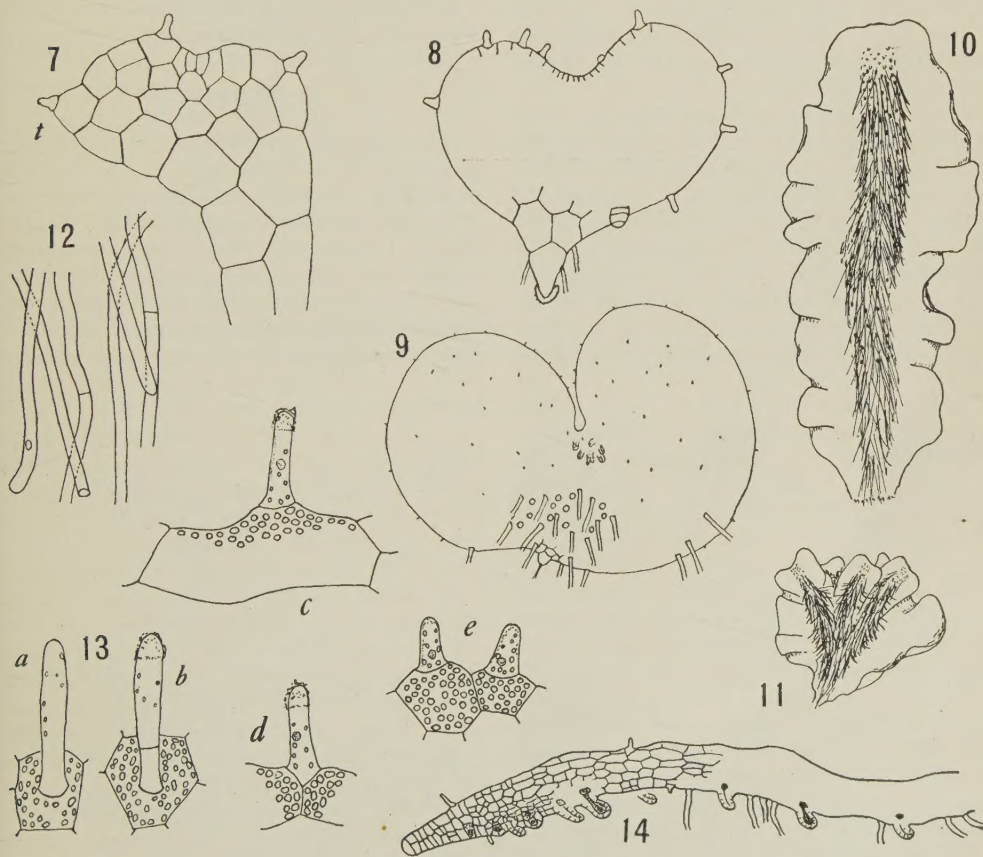
FIGS. 7-14 — Figs. 7-9, stages in the development of cordate thallus. Fig. 7, 20 days; *t*, terminal cell of filament. Fig. 8, 26 days. Fig. 9, 50 days. Figs. 10, 11, gametophytes 18 months old. Fig. 11, branched thallus with 3 apical meristems. Fig. 10, ×4; Fig. 11, ×2. Fig. 12, portion of a mass of rhizoids. Fig. 13, hairs; (*a*, *b*) from ventral surface near archegonia; (*c*, *d*) marginal hairs from old thallus; (*e*) marginal hairs from young thallus. ×280. Fig. 14, l.s. thallus 18 months old; archegonium initial and old stages.

antheridia abundantly. They were also borne in large numbers on regenerated branches of old gametophytes. Occasionally antheridia are found on filamentous outgrowths from the basal region of old gametophytes (Fig. 18). On the midrib of large old gametophytes there are often outgrowths of green cells — bulbous or disc-shaped — which may give rise to one òr two antheridia, one or two rhizoids or hairs, or a combination of these (Figs. 21-24). The disc-shaped outgrowths (Figs. 16, 21, 22) may appear to be a part of the antheridium, but the abundance of green plastids indicates that it is a part of the thallus, and the discolouring of the wall which is seen in old antheridia does not extend to this cell (Fig. 21). Döpp (1927) reported such outgrowths in several species of higher ferns.

The antheridia are globular or very slightly elongated. Young gametophytes bear smaller antheridia than old and they are perhaps less likely to be elongated (Figs. 19, 20). The basal cell is relatively large in diameter, and while it may become funnel-shaped from the pressure of the spermatogenous contents (Figs. 20, 24), this does not always happen, and it is more likely to be saucer-shaped

or disc-shaped (Figs. 19, 22, 23). The antheridium has a heavy layer of cutin. The cap cell is rather large; its behaviour at the dehiscence of the antheridium was not observed. The spermatogenous contents escaped with great rapidity. Unfortunately the detailed study of the antheridium was postponed when the gametophytes were young and the antheridia in a favourable condition for study. On young gametophytes the antheridia are normal and produce active sperms, but in our cultures the antheridia borne on old slender gametophytes and on regenerated branches did not mature or did not discharge sperms. There were a few cases in which the cap cell was divided (Fig. 25) but typically it was undivided as is usual in the higher ferns.

Archegonium

The archegonium initial (Figs. 14, 26) appeared in our material on the ventral surface in the sixth to ninth segment cut off from the meristematic initials at the notch (Figs. 14, 50). Its nucleus is larger than that of the neighbouring cells, and the cytoplasm stains deeply and contains numerous small peripheral vacuoles. The cells are small and the plastids not
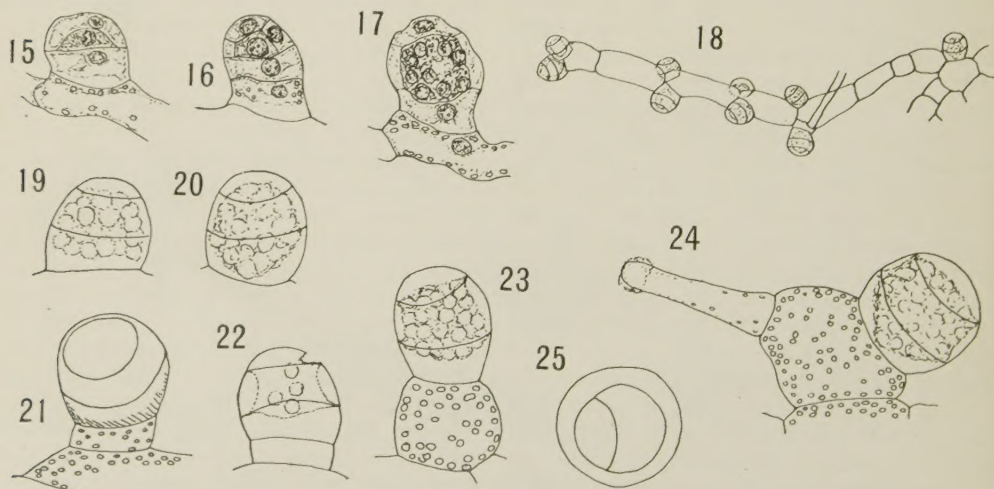

FIGS. 15-25 — Antheridium. Figs. 15-17, l.s. three stages in the development of antheridium. Fig. 18 filamentous outgrowth bearing antheridia, from base of old thallus. Figs. 19, 20, antheridia from gametophyte 50 days old. Figs. 21-25, antheridia from old gametophytes (15-18 months). Figs. 21-24, antheridia from outgrowths on the venter. Fig. 25, divided cap cell.

sufficiently preserved to be discussed in this paper. The initial divides by a wall parallel to the surface of the thallus into an outer, usually smaller, primary neck cell and a larger inner cell (Figs. 27, 50). Both cells may then divide about the same time (Fig. 27) but usually the inner divides first (Fig. 28) by a wall parallel to the surface of the thallus, cutting off a basal cell and forming the characteristic tier of three cells (Figs. 29, 31). The prothallial cells flanking the young archegonium also divide and young hairs are frequently found in this region (Fig. 31). The primary neck cell soon divides (Figs. 30, 32) forming two, then four, surface cells — the initials of the four tiers of neck cells which cover the enlarging cell below. The first wall may be the one parallel to the longitudinal axis of the thallus (Fig. 32), or the other may be formed first (Fig. 33). These cells arch above the surface of the thallus covering the rapidly growing cell beneath. The primary cell of the axial row continues to enlarge, and, pushing outward as a column, forces the neck cells to project outward in the form of a dome (Figs. 34, 35); it becomes bluntly pear-shaped and as it grows the cytoplasm becomes more vacuolate at the tip (Figs. 34, 35). The vacuolation becomes more pronounced after the division of this cell (Fig. 37) into the central cell and the neck canal cell (Fig. 36). When this division occurs late and there are as many as three neck cells in each tier, the vacuoles are very pronounced (Figs. 38, 39). The prothallial cells adjacent to the lower neck cells divide further (Figs. 38, 39) and the development of the ventral jacket proceeds. The next division in the axial row is usually that of the neck canal nucleus to form a binucleate neck canal cell (Fig. 41, *a*), which in turn is followed by the unequal division of the central cell to form a small ventral canal cell and a large egg. There seems to be some variability in the order of division, however, as one archegonium was observed in which the neck was short and the central cell prepared for division first (Fig. 40). Another archegonium was found in which the ventral canal cell was already present

as the neck canal nucleus completed its division (Fig. 42). Divisions in the neck cells do not take place simultaneously in the four tiers of cells (Figs. 36, 40, 41*a*). The most frequent divisions are at the base and tip of the elongating neck (Fig. 41*b*). About the time that the neck canal nucleus divides, the neck of the archegonium begins to curve towards the base of the thallus, and the cells in the neck usually number five for the anterior and four for the posterior side (Fig. 41). At maturity they number six and five in most archegonia (Fig. 44*a*) and the curvature is marked (Fig. 44*b*). In a few cases this was extreme and the neck lay almost parallel to the surface of the thallus with consequent distortion of the archegonial cells. Occasionally a neck is longer and straighter than those near it, but the number of neck cells is usually the same, the additional length being attained by a lengthening of cells in each tier (Fig. 45). The basal cell usually divides early (Figs. 34, 36) so that during the development of the archegonium the sections show two basal cells. They may divide again as the curvature of the neck begins, or one cell may divide (Fig. 41) giving three in longitudinal section, although one (Figs. 42, 44), or two (Figs. 43, 45), or four (Fig. 49) may be found. Whatever the appearance in longitudinal section the egg cell at maturity rests on three or four basal cells.

The lowest neck cells divide periclinally at maturity (Figs. 44, 45). This accommodates the enlarging venter and some archegonia become very broad. The prothallial cells adjacent to the egg and basal cells divide further, completing the formation of the ventral jacket layer (Figs. 44*a*, 45) of which only the basal cells have been derived from the archegonium initial. The free end of the archegonium becomes bulbous, perhaps expanding under the pressure of the vacuole which is so conspicuous at the tip of the neck canal (Figs. 43-45). The neck cells directly over this region stretch and become relatively thin (Figs. 44*a*, 45). In our material most of the archegonia did not open. Why this is so is not clear since the archegonia were
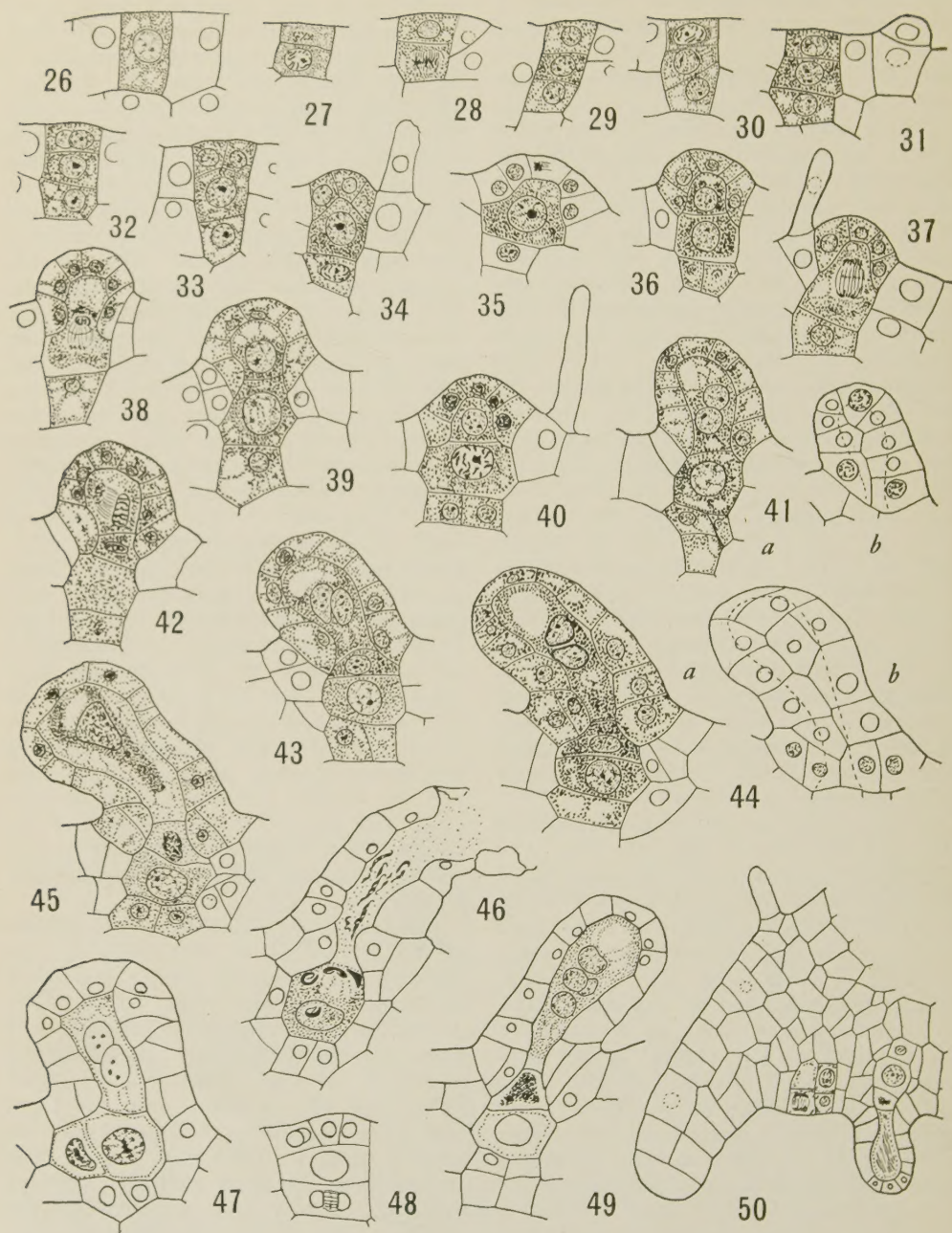
FIGS. 26-50 — Archegonium. Fig. 26, archegonium initial. Figs. 27-44, stages in development. Figs. 31, 34, 37, 40, young archegonium and hair. Fig. 41 (a, b), adjacent sections; (b), cells at base and tip about to divide. Fig. 44, (a, b), adjacent sections. Fig. 45, mature archegonium, ventral and neck canal cells degenerating. Fig. 46, fertilization. Figs. 47, 48, anomalous archegonia on thallus 18 months old. Fig. 49, mature archegonium, with 4 neck canal nuclei. Fig. 50, l.s. of thallus 18 months old.

structurally complete. If any archegonia opened, several would be found open on that gametophyte. Occasionally fertilization was realized (Fig. 46) and we obtained a few sporophytes. Several archegonia were found in which the neck canal nucleus had divided twice. In some of these only three nuclei were visible, the middle being larger than the other two. In one section of the archegonium (Fig. 49) four nuclei were counted, and it is possible that there had been four in the other instances.

As the gametophytes grow older and more massive they show a number of deviations from the above account. The last cell formed in the axial row often produces a ventral canal cell and egg of nearly equal size. This wall is often strongly curved so that the lower part of the ventral canal cell is in a cup-shaped depression in the egg cell. (Caution should be used in determining whether the egg and ventral cell are of equal size or not, as this effect can also be obtained in cutting sections at the edge of a thick midrib.) Two eggs may be formed in the venter (Fig. 47); two ventral canal cells may be present, side by side or one above the other; walls in the neck may come in irregularly. All these may be considered as old-age phenomena, or adjustments made to the increasing size of the thallus. Occasionally adjacent cells produce each an archegonium (Fig. 50). These grow to maturity with a full complement of cells except for that portion of the venter where the two egg cells touch.

Discussion

In most of the 19th-century literature the position of *Stenochlaena* was considered to be with the *Blechnum* group. Diels (1902) placed it in the Blechnineae of his subfamily Aspideae. In more recent classifications we find it in widely separated groups: Christensen (1938) placed it with *Acrostichum* and *Neurocallis* in a group of " Acrostichoid genera probably derived from the Pteridoideae "; Ching (1940) put it in the tribe Aspideae of his family Aspidiaceae; Copeland (1947) agrees with the earlier point of

view and has placed it as the last genus in his family Blechnaceae; and Holttum (1949) has assigned it to the subfamily Pteridoideae of his family Dennstaedtiaceae.

The early stages of the gametophyte of *S. palustris* are like those of *Blechnum* spp. as described by previous investigators, and as we found them in our cultures of *B. spicant* (L.) Wither. and *B. buchtienii* Rosenst., an account of which also appears in this same journal (Stokey & Atkinson, 1952). The mature gametophyte of *S. palustris* is strikingly like that of *B. spicant* and cultures of the two could easily be confused, but it does not resemble that of *B. buchtienii* so closely. The young stages are alike particularly in the appearance of the hair on the terminal cell of the young filament; the later hairs are of the same type and vary only in abundance; the old gametophytes have the same tendency to develop a heavy midrib with an occasional forking of the midrib; they both show the same dense growth of rhizoids with occasional septations in the rhizoids. The notable difference between the two genera is in the form of the antheridium; that of *Stenochlaena* is globular or at most slightly elongated, while that of *Blechnum*, so far as is known, is of a vase-shaped elongated type with a columnar rather than a funnel- or disc-shaped basal cell. This is a much less common type than the globular but is found in various other genera (Stokey, 1951). We do not know enough about the general occurrence of this type in general, and in particular in the *Blechnum* group and in the numerous species of *Blechnum*, to appraise its significance. The characters which *Stenochlaena* has in common with *Blechnum* are found in a considerable number of genera of the higher ferns. The development of a filament in which the growth is checked by a terminal hair is found in the *Dryopteris* group and in several species of *Asplenium*, as well as in other genera (Döpp, 1927). The type of hair found in both genera is the most common type in the higher ferns. We do not know to what extent general habit and aspect may indicate a genetic connection — probably in very few cases, if any.

The gametophyte of *Stenochlaena* has much less in common with that of *Acrostichum aurem* L. as described by Schumann (1915) or with that of *A. speciosum* Willd. than with *Blechnum*. We have had *A. speciosum* in cultivation for 18 months and an account of our investigation will appear shortly in this journal. In the first place, the gametophyte of *Acrostichum* is naked and does not bear hairs either when young or at any later stage; in the second place, the gametophyte of *Acrostichum* is of the asymmetrical type with a definite lateral meristem. The asymmetry is not temporary as in *Stenochlaena* but is persistent. A wedge-shaped apical cell is not formed, and there exists from an early stage a lateral marginal meristem of which the distal products are so much greater than the proximal that it results in a very unequal development of the wings (Stokey, 1951). *Acrostichum* is an extreme case of the asymmetrical type. There is also a difference in the form of the antheridium; that of *Stenochlaena* is shorter and does not have the peculiar lop-sided cap cell of *Acrostichum*, although it occasionally shows a slight tendency towards a conical cap, which is common in *Acrostichum* in a much more pronounced form.

In considering the question of affinity, the asymmetrical habit of *Acrostichum* seems to us to be a character of considerable weight; the naked thallus of *Acrostichum* and the peculiar antheridium have less weight but are good characters. While the evidence from the gametophyte is against an affinity with *Acrostichum*, it could quite as easily ally it with *Dryopteris* or *Asplenium* because of the similarity of the antheridium, if there were adequate reasons for such an interpretation. Since the gametophyte of *Stenochlaena* does not share the peculiar characteristics of *Acrostichum* — lack of hairs, asymmetry of thallus, and peculiar antheridium — the evidence from the gametophyte is in harmony with the recent work of Mehra and Chopra (1951) on the anatomy of *S. palustris*. The gametophyte of *Stenochlaena* has much in common with that of *Blechnum*, known at present from relatively few species, but the characters which they have in common may also be found in other groups; the real difference is in the antheridium, but we know its character in only two species of *Blechnum*, both of the *Lomaria* section.

Summary

Germination of the spores occurred on water in 7-8 days. A few spores retained their viability for 18 months. A filament of 4-7 cells develops with a papillate hair on the terminal cell, and then plate formation begins by longitudinal divisions in cells behind the tip. The apical cell is lateral for a short time but is soon shifted to a central position. The mature thallus is cordate with many papillate hairs on margin and surface. Antheridia appeared in 20 days after planting spores, archegonia in 5-6 weeks. The antheridia are globular or very slightly elongated. A detailed account is given of the archegonium from initial to fertilization of egg. Occasionally there are four nuclei in the neck canal. A discussion is given of the affinity of *Stenochlaena*. The gametophyte has much more in common with that of *Blechnum* than with that of *Acrostichum*.

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THE GAMETOPHYTE OF *BLECHNUM SPICANT* (L.) WITHER. AND *B. BUCHTIENII* ROSENST.

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In connection with the investigation of the gametophyte of *Stenochlaena palustris* (Burm.) Bedd. (Stokey & Atkinson, 1952) it has seemed desirable to assemble what is known about the gametophyte of *Blechnum* and to examine the gametophytes of the two species which were available. These were raised from spores, of which those of *B. spicant* (L.) Wither. were collected by the junior author on the Olympic Peninsula, State of Washington, in August 1950. We are indebted to Miss Edith Scammon for the spores of *B. buchtienii* Rosenst. which she collected in Costa Rica in April 1951.

There are accounts of four species of *Blechnum* of which the gametophyte has been investigated more or less in detail, chiefly in the early stages: *B. punctulatum* Sw. by Stübner (1882); *B. occidentale* L. by Lampa (1901); *B. brasiliense* Desv. by Jung (1927); and *B. spicant* by Karpowicz (1927) and by Döpp (1927).

Most of the investigation, including that of the antheridium, was made from living material. Microtome sections were used for the study of the thallus and of the archegonium with the methods described for *Stenochlaena*. Smears of the antherozoids were fixed over osmic acid fumes, stained in Haidenhein's haematoxylin, and mounted in balsam.

The spore and early stages of development of the gametophyte of *B. spicant* have been described by both Karpowicz and Döpp, so we shall describe only those of *B. buchtienii*. The spore of *B. buchtienii* is of the bilateral type, with a dark-brown coat irregularly rough (Fig. 1). The average size is 52×42 μ, but there is considerable variation because the spore is often more or less rounded. After germination of the spore there develops a filament which consists usually of 4-5 cells (Fig. 2). In most cases the growth of the filament is checked by the formation of a papillate hair on the terminal cell. In such cases the plate (Figs. 3-6) arises in the manner described for *Stenochlaena*, and the wedge-shaped apical cell arises laterally, but by the growth of adjoining cells is shifted to a position near the tip of the longitudinal axis. In some cases the filament does not end in a papillate hair, and then the apical cell is formed by the terminal cell of the filament (Fig. 7). A further development of this type is seen in Fig. 8 with the development of hairs on cells formed from the terminal and second cells.
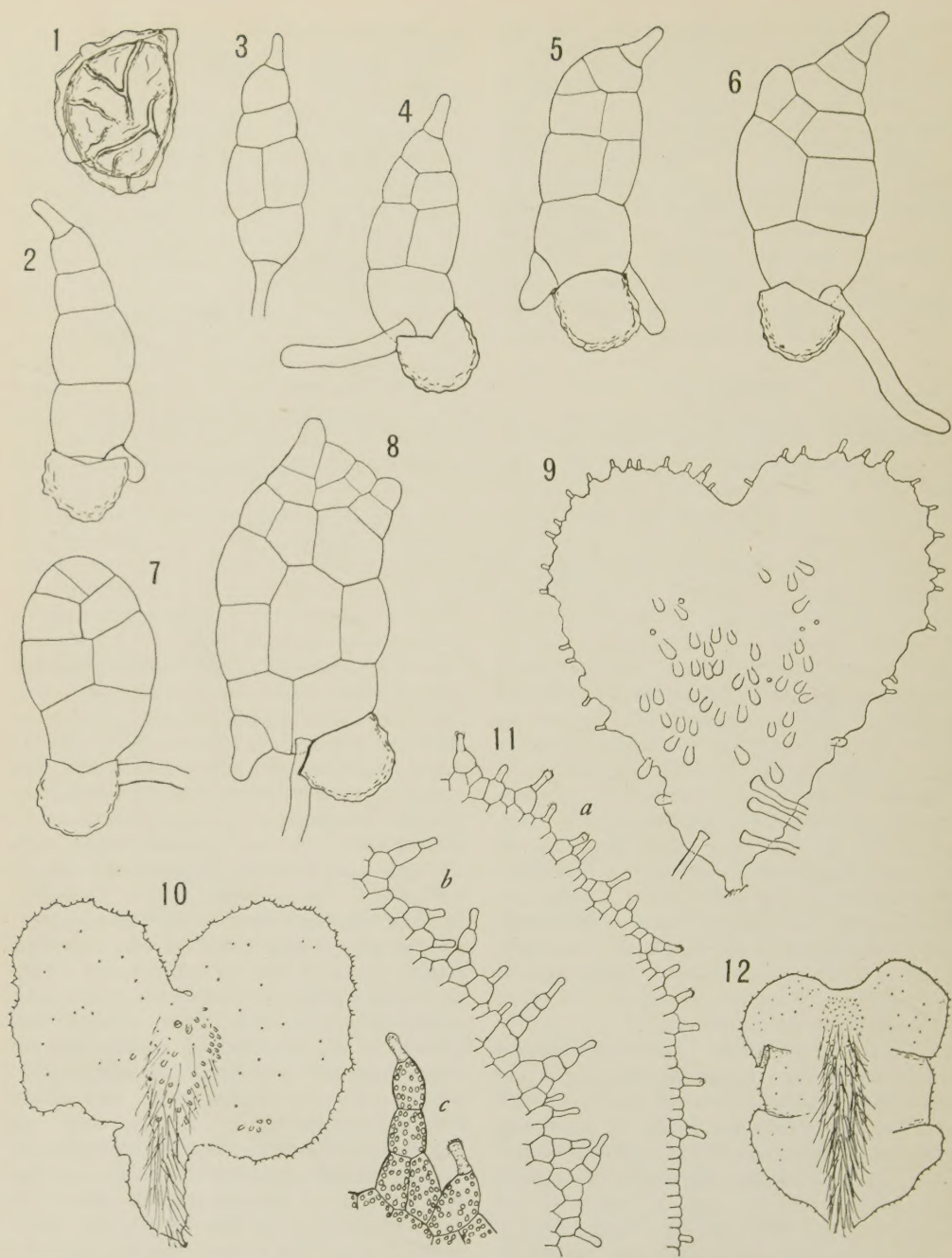
FIGS. 1-12 — *B. buchtienii.* Fig. 1, spore ×350. Fig. 2, filament ending in terminal hair. Figs. 3-6, examples of development of plate from filament with terminal hair. Figs. 7, 8, gametophytes in which terminal cell gives rise to apical cell. Fig. 9, gametophyte with antheridia. Fig. 10, gametophyte with antheridia and archegonia. Fig. 11, margin of thallus; (*a*), margin from near apex and young portion of wing; (*b, c*), margin from older portion of wing. (*a, b*), ×67, (*c*) ×125. Fig. 12, thallus 7 months old.

The cordate stage is soon attained and the gametophyte of both *B. spicant* and *B. buchtienii* is broadly cordate at maturity — the stage at which both antheridia and archegonia have developed — with papillate hairs on both surfaces and more abundantly on the margin.

The previous accounts of *B. spicant* do not describe the character of the thallus in old cultures. It shows nothing unusual in habit until about 6 months old and then it tends to become unusually large with a thick midrib and a very dense growth of reddish-brown rhizoids. At 16 months the midrib may be 8-12 cells thick, very much rounded on the ventral side and forming a long depression between the wings on the dorsal side (Fig. 38). The midrib may branch in the case of large old gametophytes and form several meristem regions bearing archegonia. The cultures were strikingly like those of *S. palustris*.

The gametophytes of *B. buchtienii* were not in culture as long as those of *B. spicant*. The thallus was always thinner (Fig. 30) and even at 8 months gave no suggestion of the heavy thallus of *B. spicant* at that age, although the thallus continues to grow and elongates considerably (Fig. 12). In general appearance they were strikingly different because of the toothed margin which characterized the plate (Figs. 9, 10). The hairs are of the same type as those of *B. spicant*, but they are much more numerous on the margin where they are borne on more or less extended projections several cells beyond the general contour of the thallus (Fig. 11, *a-c*); (*a*) shows a region near the apex where the hairs have just begun to develop and the margin is smooth extending partly into a wing; (*b, c*) show older regions where the tooth-like projections have become more prominent; (*c*) shows the thallus-like structure of the projection and that it is not a case of a multicellular hair. This type of margin was not described for *B. punctulatum, B. occidentale,* or *B. brasiliense*. If it had been present it surely would have been noted even in the short-lived cultures, as it is a character which shows at an early age. It is evidently not a genus character. It has been noted in the gametophytes of other ferns not considered to be closely related to *Blechnum*: Karpowicz described it for *Phegopteris polypodioides* Fèe [*Dryopteris polypodioides* (Raddi) C. Chr.], and Döpp for *Asplenium ruta muraria* L. and *Aspidium aculeatum* Sw. [*Polystichum aculeatum* (L.) Schott].
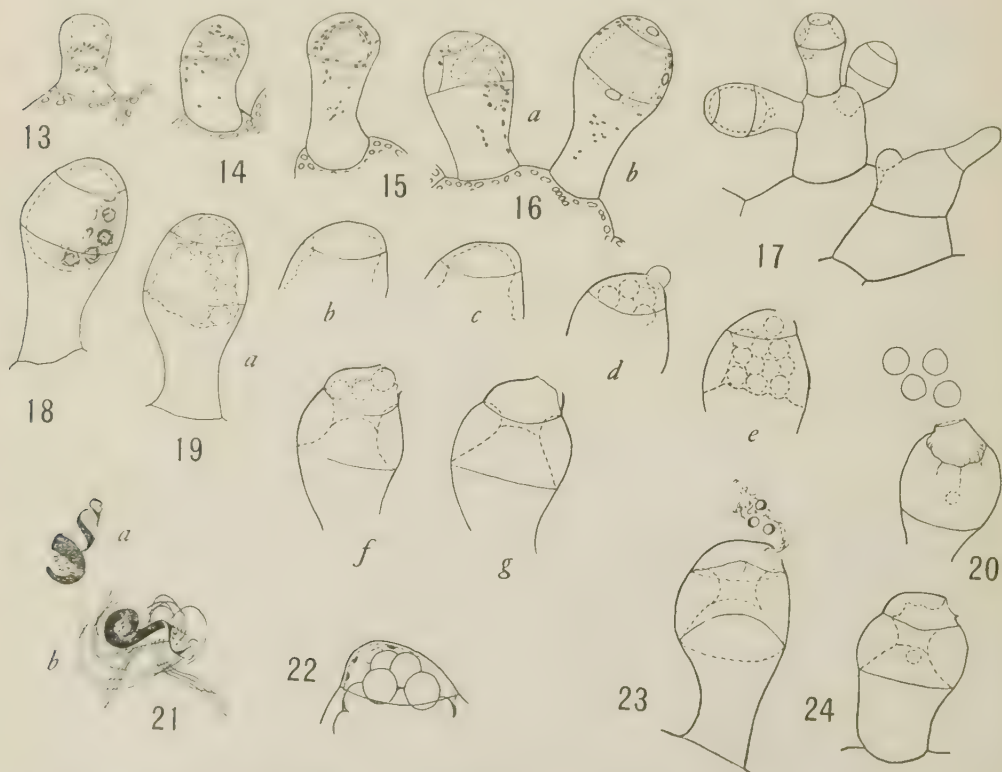
Antheridium

The antheridium initial (Fig. 13) arises from the surface of the thallus as a protuberance which is soon divided by a cross wall into two unequal cells (Fig. 14). The larger, lower cell becomes the basal cell of the antheridium and the upper cell divides (Fig. 15) into an outer wall cell and an inner dome-shaped cell. The wall cell divides (Fig. 16*a*) to form the cap cell, and the inner cell gives rise to the spermatogenous mass (Figs. 15, 16, 18) dividing four or five times to produce 32 or 64 antherozoids. These depress the upper wall of the basal cell, but not sufficiently to make it funnel-shaped. The basal cell during development grows in length and forms a pronounced stalk-like cell which often curves backward so that the antheridium may lie nearly parallel to the surface of the thallus. Usually only one antheridium arises from a prothallial cell, but there may be two formed (Fig. 16), or, in the case of ameristic male prothalli, more than two (Fig. 17). The plastids in the antheridial initial and in the succeeding stages are fewer and smaller than those in the adjacent thallus cells, so that the antheridium when mature appears colourless. The coiled antherozoids are then clearly visible through the lateral walls (Fig. 18) which appear to be of uniform thickness. When the antheridia come in contact with water they dehisce, and the spermatogenous cells containing the coiled antherozoids are extruded into the water where they remain for the short time necessary for each to free itself from the surrounding membrane.

It is difficult to observe the actual opening of the antheridium as it takes place so quickly, but a tiresome vigil revealed the following sequence of events leading up to, and during dehiscence of,

two antheridia, with corroborative evidence from other antheridia in which the entire process was not observed. Shortly after coming in contact with water, the upper wall of the basal cell begins to push the spermatogenous mass upward so that the imprint of the individual cells becomes evident on the walls surrounding them (Fig. 19, *a-c*). At the same time the lateral walls swell and press inward so that the spermatogenous cells become compressed and the walls between them are no longer visible (Fig. 19*e*). The cells appear to be spherical and as the pressure continues they seem to overlap. The cap cell soon develops a small beak at one side (Fig. 19*c*) into which the nucleus and the few plastids present are pushed (Fig. 22). Suddenly the cuticle covering the beak snaps, and there is a quick expulsion of a small amount of material (probably the nucleus and plastids mentioned above, as vesiculated plastids are sometimes seen sticking to the outside of half-opened antheridia). As soon as the cuticle snaps one of the spermatogenous cells moves toward the beak, and pushes smoothly through a very delicate membrane (Fig. 19*d*) which seems to close partially behind it. This is suggested by the rise and fall of the cuticle with the expulsion of the first 10-15 cells. The side and basal walls of the antheridium continue to expand inwards, with the release of pressure and the intake of water, forcing the spermatogenous cells through the pore in rapid succession until some 20 have been extruded. Then the escape of the spermatogenous cells slows down, and often at the time the last two or three are extruded all those outside have been released from

FIGS. 13-24 — Antheridium. *B. buchtienii*. Figs. 13-16, development of antheridium. Fig. 17, portion of ameristic male thallus; 3 antheridia borne on one cell, hair and young antheridium on another. Fig. 18, mature antheridium. Figs. 19, 20, 22, stages in dehiscence. Fig. 21, free-swimming antherozoid. ×700. Figs. 23, 24, antheridia after dehiscence.

their membranes. By this time the pore has become established (Fig. 19, *f*, *g*) and the cap cell has lost its original shape. It appears puckered and being transparent can easily be overlooked if the antheridium is observed from above, although it is very clear in lateral view. The empty walls eventually come to occupy most of the interior of the antheridium and apparently continue to exert pressure, as vesiculated plastids and granular material can be found at the pore (Fig. 23) long after the antheridium is emptied. This is presumably some of the protoplasm from the ruptured cap cell (Fig. 24) as has already been suggested by Hartman (1931, p. 263).

The swimming antherozoid is coiled (Fig. 21*a*) and moves smoothly through the water indicating that it does not carry the spermatogenous cell membrane as a vesicle. In fixed material the structure can be more clearly seen (Fig. 21*b*).

Archegonium

The archegonium develops in the usual manner (Figs. 26-29, 31-35) from an initial appearing in our material in the 7th or 8th segment cut off from the apical meristem (Fig. 30). The neck canal cell may divide before the ventral canal cell is formed (Fig. 33), or after (Fig. 34). The neck of the mature archegonium consists of 4 or 5, sometimes 6, cells, those covering the tip being stretched thin (Figs. 35, 37). The ventral jacket is well developed; the axial row consists of the egg, ventral canal cell, and binucleate neck canal cell which is vacuolated at the tip. The ventral canal cell and neck canal cell degenerate, the neck opens, and fertilization takes place readily as evidenced by the production of numerous sporophytes. Anomalous archegonia were found on old gametophytes (Fig. 36).

Discussion

The five previous accounts together with our investigation indicate that after the development of the usual filament the formation of the plate may be brought about in either of two ways: (a) the terminal cell may end in a papillate hair and then the wedge-shaped apical cell arises laterally from one of the cells behind the tip; (b) the terminal cell does not bear a hair and it divides in such a way as to produce a wedge-shaped apical cell which is approximately terminal from the beginning. There are variants in both methods in the sequence of divisions leading to the formation of the apical cell.

Stübner found that both methods, (a) and (b), occurred in *B. punctulatum*; Lampa reported the same for *B. occidentale*. Jung stated that in *B. brasiliense* the " protonema " develops as in other members of the Polypodiaceae and the terminal cell becomes the apical cell. (Apparently he worked with a small amount of material, perhaps not enough to show what variation may exist.) Karpowicz found both types in *B. spicant*, but Döpp said that in his material the " protonema " always ended in a papilla and the apical cell was always developed from the cells behind the tip. We found both methods in *B. spicant* and *B. buchtienii*, but (a) in which the terminal cell ends in a hair is much the more common, and, in fact, is the characteristic method, just as in *Stenochlaena*.

The papillate hair — the most common type in the higher ferns — is present in all five species in greater or less abundance. According to the accounts of *B. spicant* there is considerable variation in their number in this species. *B. buchtienii* is the only species in which hairs are borne on tooth-like processes on the margin.

The antheridium in *B. spicant* and *B. buchtienii* is of the elongated type with a columnar basal cell. Both Karpowicz and Döpp noted this and gave figures of the antheridium of *B. spicant*. The structure of the antheridium for *B. punctulatum* and *B. occidentale* was not given. Jung stated that in *B. brasiliense* the structure agreed with that of the " einheimischer " species in his investigation, and is presumably of the usual globular type. His results for *B. brasiliense* in regard to the antheridium and also the behaviour of the young filament do not agree with the results from the other four species. It should be noted
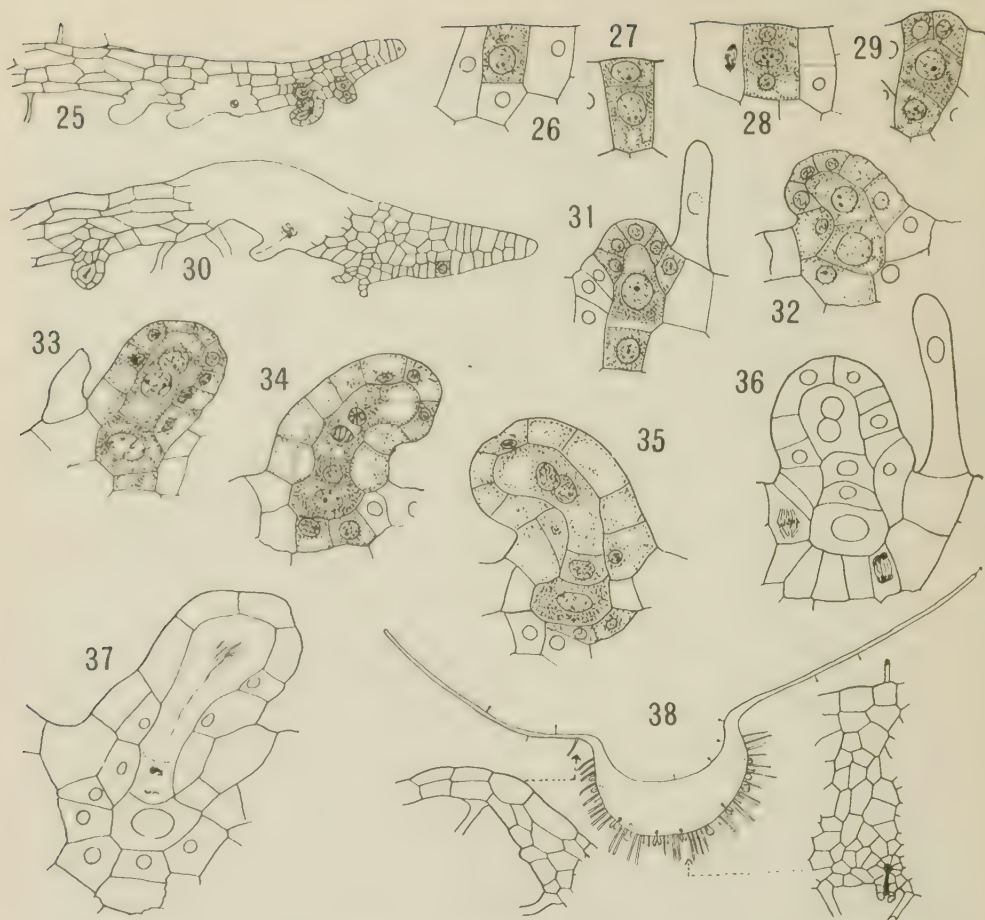
FIGS. 25-38 — Fig. 25, l.s. prothallus 7 months old.   Figs. 26-29, 31-35, development of arche-gonium.   Fig. 30, l.s. gametophyte 15 months old.   Fig. 36, anomalous archegonium.   Fig. 37, mature archegonium.   Fig. 38, c.s. gametophyte 15 months old.   Figs. 26, 27, 29-31, 33, 36, *B. spicant*; Figs. 25, 28, 32, 34, 35, 37, *B. buchtienii*.

that the latter species belongs to the section Eublechnum and the other four to Lomaria.   It is desirable to have more information about the gametophyte of *Blechnum* and especially of species in the Eublechnum section.

Summary

Germination of spores of *B. spicant* and *B. buchtienii* resulted in a 4-5-celled filament in which the terminal cell regularly ended in a hair, and the apical cell was then developed from a cell behind the terminal cell.   In a few cases in which the terminal cell had not borne a hair the apical cell was developed from the terminal cell.   Papillate hairs are common in both species on surface and margin; in *B. buchtienii* the marginal hairs were borne on tooth-like processes.   The thallus at maturity is broadly cordate; that of *B. spicant* may become very large at 6-16 months with a midrib 8-12 cells thick which may branch; that of *B. buchtienii* is of a less heavy type.   The antheridium is much elongated and the basal cell is columnar, never funnel-shaped; the de-

hiscence was studied in detail. The archegonium showed no unusual features in its development.

Part of this investigation was carried out by the senior author at the Marine Biological Laboratory, Woods Hole, Mass. The junior author wishes to express her gratitude to the Biology Department, Amherst College, Amherst, Mass., for certain materials and use of apparatus.

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THE EMBRYOLOGY OF *LILAEA SUBULATA* H.B.K. WITH A DISCUSSION ON ITS SYSTEMATIC POSITION

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Introduction

The monotypic genus *Lilaea* comprises a single species, *L. subulata*, which is found in many parts of the western coast of North and South America. There is a difference of opinion regarding its systematic position. Bentham and Hooker[1] (1883) and Engler and Prantl (1889) include four genera — *Lilaea*, *Triglochin*, *Scheuchzeria* and *Tetroncium* — in the family Juncaginaceae (= Scheuchzeriaceae). Hieronymus (1892) and Schumann (1892) place *Lilaea* in a separate family, Lilaeaceae[2]. This has been supported by Hutchinson (1934) who, however, proposes an alternative name,

Heterostylaceae, " for anyone who may quite naturally object to the use of a family name so similar to that of Liliaceae ".

Earlier investigations on *Lilaea subulata* by Hieronymus (1892) and Campbell (1898) are old and incomplete, and, therefore, a reinvestigation was suggested by Prof. P. Maheshwari. He very kindly passed on to me the material collected by him in Southern California during 1947 when he was visiting some of the Universities in the United States. Some material was later sent to him by Prof. H. F. Copeland[3] (Sacramento, U.S.A.) which was also put at my disposal.

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1. See also Hooker (1894).
2. Quoted from Campbell (1898).

The usual methods of dehydration and imbedding were followed. Sections were cut 5 to 15 μ thick and stained in iron-alum haematoxylin as well as in safranin and fast green, both of which gave good results. Acetocarmin smears of the anthers were also tried.

Previous Work

Hieronymus (1892) described the histology of the vegetative organs of *Lilaea*, particularly the roots and leaves. A few years later Campbell (1898) gave a more detailed account of its morphology and life history. According to him the mature pollen grains are two-celled, and the primary sporogenous cell of the ovule usually divides into three cells, of which the middle one develops into the embryo sac. He reports an increase in the number of nuclei and the development of cellular tissue in the embryo sac before fertilization, and suggests that it may be a case of reversion to a primitive condition. The endosperm is nuclear and the radicle is said to originate laterally.

External Morphology

The plant grows in aquatic or marshy places. It has a short rhizome with numerous fibrous roots and radical leaves which have an open sheath at the base.

The young inflorescence arises from the base of the rhizome and is completely hidden among the leaf bases. It is a pedunculate spike with numerous pairs of male and female flowers arranged spirally and terminated by a male flower (Fig. 1). Fig. 4 shows one such pair, Fig. 3 represents a longitudinal section of the terminal portion of the spike and Fig. 2 is a cross section through its lower part. Numerous air chambers occur in the inflorescence axis.

Each male flower is subtended by a perianth segment[4] and consists of a single stamen with a two-lobed anther (Fig. 7) mounted on a very short stalk. The terminal male flower is usually twice as large as the laterals (Figs. 5, 6).

The female flowers are of two types. Two of these, which are laterally situated at the base of the inflorescence axis, are very conspicuous because of their extremely long styles (Fig. 1). The rest of the flowers, which are spirally arranged on the upper part of the axis, have much shorter styles and are only about one-sixth the size of the two basal flowers (Figs. 1, 3)[5].

Campbell (1898) considers the two long-styled flowers " probably represent shoots of the same nature as the innovations which occur in the larger plants, in addition to the shoots formed in the axils of the leaves ".

According to Jepson (see Hutchinson, 1934, p. 41), the spikes may be of two types: (i) those consisting exclusively of unisexual flowers, and (ii) those with hermaphrodite flowers in the middle, female below and male above, all excepting the female arising in the axil of a bract. According to Hutchinson (1934) the so-called bract is a single perianth segment.

All female flowers consist of a single carpel with a hollow style and without any perianth lobe. The stylar canal is very narrow at the lower end. The stigma is papillate. Fig. 8 shows a longitudinal section of a short-styled flower while Figs. 9-15 represent transverse sections of a slightly older carpel than that shown at levels approximately *a*, *b*, *c*, *d*, *e*, *f* and *g*. Fig. 16 shows the glandular lining of the stylar canal at its stigmatic end.

Vascular Supply of Flower

The inflorescence axis shows 6-7 bundles arranged in a ring (Fig. 2). Each pair of male and female flowers receives a single strand which soon bifurcates so as to give off one branch to each (Fig. 3). The trace to the male flower bifurcates again with one branch going to the perianth lobe and the other into the connective (Figs. 3, 7). The single trace going to the female flower (Fig. 9) branches at the base of the ovary

4. Campbell (1898) describes it as a bract and considers it to be the equivalent of the leaf at the base of the main shoot.

5. I have not observed any intermediate grade between the long-styled and the short-styled flowers as described by Campbell (1898).
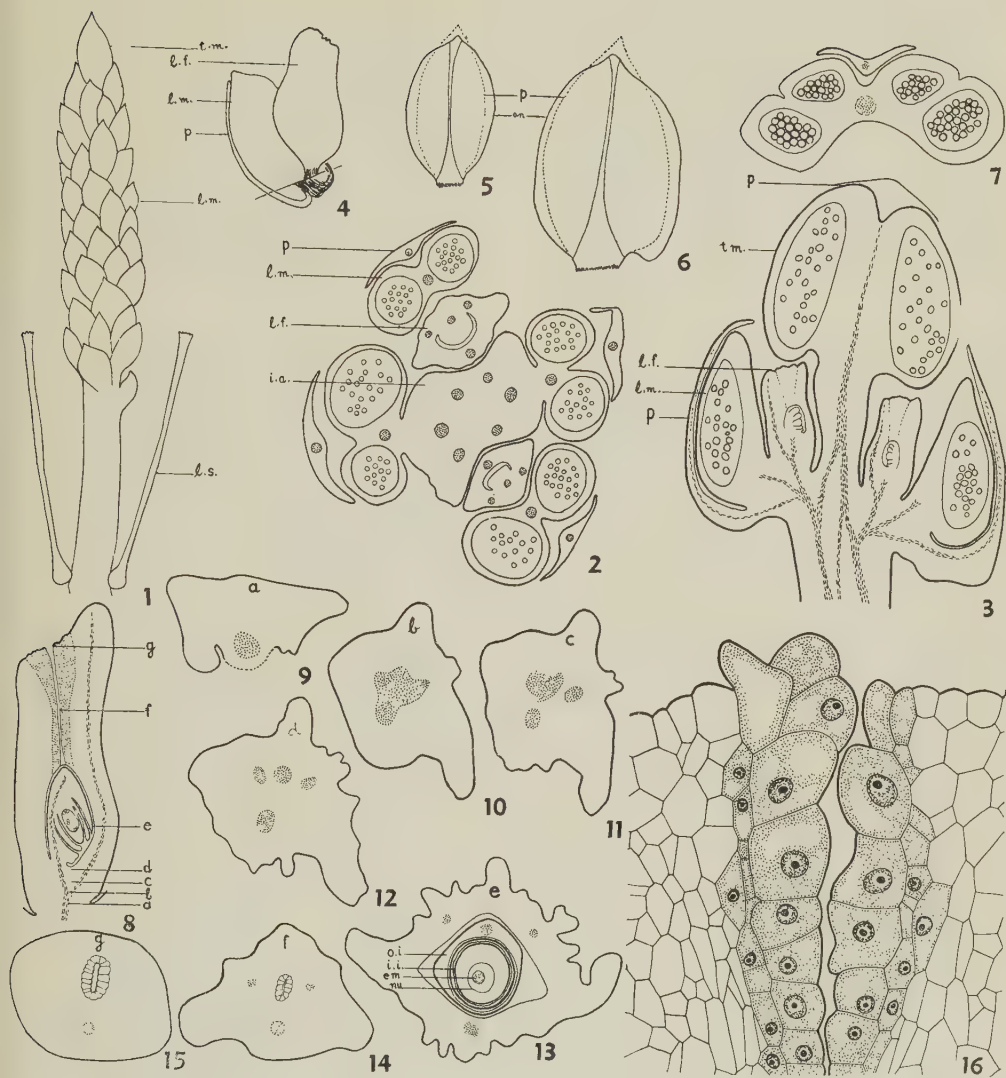
Figs. 1-16 — Morphology of the inflorescence and male and female flowers; an = anther, em = embryo, $i.a.$ = inflorescence axis, $i.i.$ = inner integument, $l.f.$ = lateral female flower, $l.m.$ = lateral male flower, $l.s.$ = long-styled female flower, nu = nucellus, $o.i.$ = outer integument, p = perianth and $t.m.$ = terminal male flower. Fig. 1, inflorescence with groups of male flowers completely covering the short-styled female flowers; note the two laterally situated long-styled female flowers at the base. ×5·5. Figs. 2, 3, t.s. of lower and l.s. of terminal portion of inflorescence respectively. ×31, ×48. Fig. 4, a pair of lateral flowers, one male and the other female. ×24. Figs. 5, 6, dorsal view of lateral and terminal male flowers respectively. Fig. 7, t.s. male flower showing the four pollen sacs and single perianth segment. ×48. Fig. 8, l.s. carpel at mature embryo sac stage. ×48. Figs. 9-15, transverse sections of a slightly older carpel at levels a-g. ×48. Fig. 16, l.s. stigma showing glandular lining of stylar canal. ×310.
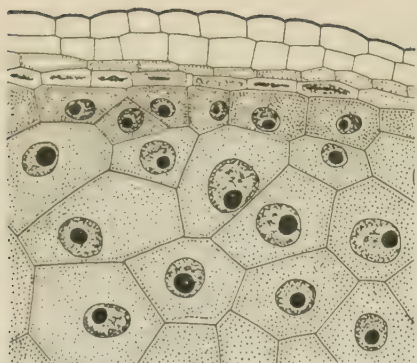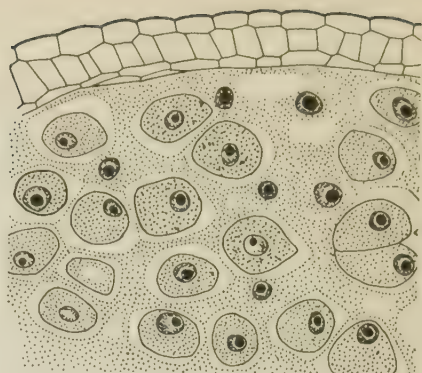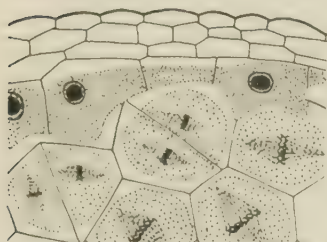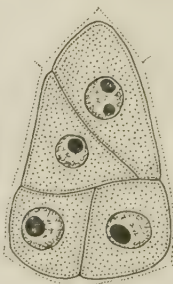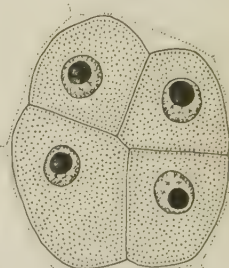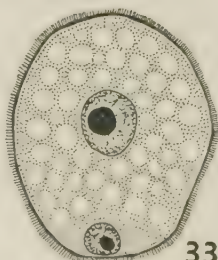
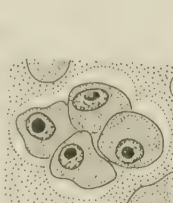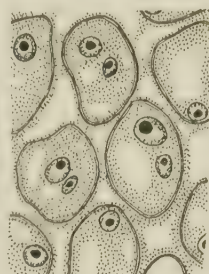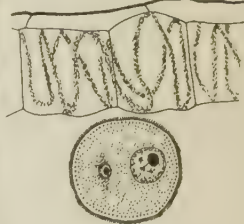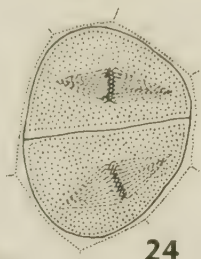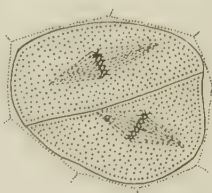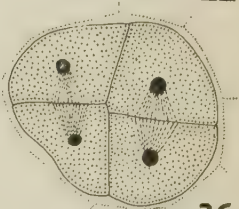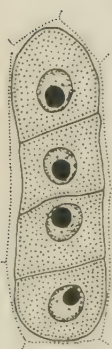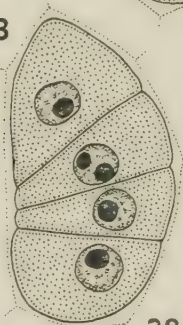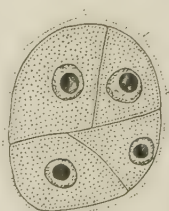Fig. 10). One of the branches, the dorsal bundle, reaches up to the stigma (Fig. 15). The other branch divides once again to give rise to two ventral bundles (Fig. 11). The ovular trace (Figs. 12, 13), which is derived from one of them, ends in the chalaza. The two ventral bundles continue upward into the style ending just below the stigma (Fig. 14).

Microsporogenesis

The youngest anther in my material showed well-differentiated microspore mother cells surrounded by the tapetum, two middle layers, the endothecium and the epidermis (Fig. 17). Out of the two middle layers, the inner gets crushed and disorganized during the enlargement of the tapetal cells and microspore mother cells. The outer layer persists until the pollen grains have become two-celled, but by the time they are mature this also disorganizes and disappears (Fig. 22). The tapetal cells remain uninucleate and take a denser stain as compared to the microspore mother cells. Their walls break down when the latter are undergoing meiotic divisions (Fig. 18) and the protoplasts and nuclei wander in between the developing tetrads giving rise to a true periplasmodium (Figs. 19, 20) corresponding to the Triglochin type of Clausen (1927). As the pollen grains mature it is gradually consumed (Fig. 21) and nothing is left of it in the mature anther (Fig. 22).

According to Campbell (1898) " the tapetal cells encroach upon the sporogenous area, and there are cells which are *intermediate in character* between the perfect sporogenous cells and those of the tapetum ". He considers these intermediate cells to be potentially sporogenous and adds that " the sporogenous cells after separation are imbedded in a nucleated mass of protoplasm derived from the tapetal cells and the imperfect sporogenous ones ". It is possible that he mistook the protrusions of the tapetal protoplasts for the so-called " imperfect sporogenous cells ".

The divisions of the microspore mother cells are successive as in the majority of the monocotyledons (see Maheshwari, 1950). During the heterotypic division a cell plate is laid down which separates the two daughter nuclei (Fig. 23). During the homotypic division the two spindles may lie in varying positions resulting in microspore tetrads which may be isobilateral (the most usual condition), linear, T-shaped or sometimes irregular (Figs. 24-31). Such variations in the form of the microspore tetrads have also been reported in *Nicolaia*, *Musa*, *Butomopsis*, *Habenaria*, *Laurus* (see Maheshwari, 1949) and *Ottelia* (Islam, 1950).

Male Gametophyte

As the microspore enlarges and a large vacuole appears in it, the centrally placed nucleus takes up a position near the wall (Fig. 32). Here it undergoes a mitotic division and produces a large vegetative cell and a much smaller generative cell separated by an ephemeral membrane (Fig. 33). Soon the generative cell moves up to a more central position and at the same time assumes a spindle-shaped appearance (Fig. 34). Frequently the vegetative as well as the generative

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FIGS. 17-35 — Microsporogenesis and development of male gametophyte. Fig. 17, l.s. part of anther lobe at microspore mother cell stage. ×590. Fig. 18, same, showing first and second reduction divisions and amoeboid projections of the tapetal protoplasts. ×590. Fig. 19, microspore tetrad surrounded by periplasmodium. ×590. Fig. 20, young microspores embedded in periplasmodium. × 590. Fig. 21, two-nucleate pollen grains with traces of periplasmodium. × 590. Fig. 22, mature pollen grain with part of anther wall. ×590. Fig. 23, microspore mother cell in telophase of Meiosis I. ×1100. Figs. 24, 25, dyads of microspores in metaphase of Meiosis II. ×1100. Fig. 26, end of Meiosis II. ×1100. Figs. 27-31, microspore tetrads in various dispositions. ×1100. Fig. 32, uninucleate pollen grain. ×1100. Fig. 33, two-celled pollen grain with the generative cell separated by an ephemeral cell plate. ×1100. Fig. 34, mature two-celled pollen grain with spindle-shaped generative cell. ×1100. Fig. 35, same, showing probable division of the generative nucleus. ×1100.
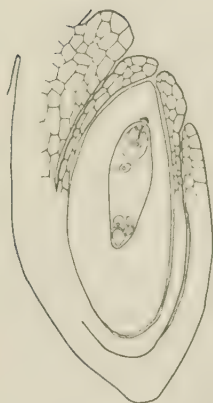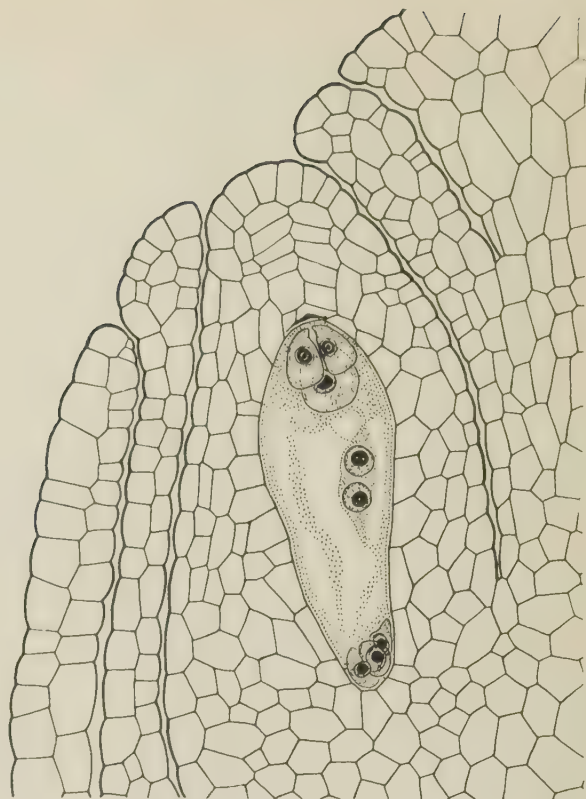
FIGS. 36-40

nuclei stain with the same intensity and, except for its smaller size and surrounding sheath of cytoplasm, the latter is almost indistinguishable from the former.

The generative nucleus does not divide in the pollen grain, which is only two-celled at the shedding stage as also observed by Campbell (1898). In my material one pollen grain showed the generative cell in division (Fig. 35) but this was probably an abnormality of no special significance. As a rule, in the order Helobiales the pollen grains are shed at the three-celled stage (see Schnarf, 1939; Maheshwari, 1949). *Ottelia* (Islam, 1950), *Zannichellia* (Campbell, 1897), *Triglochin* (Wulff, 1939) and *Lilaea* (Campbell, 1898; present work) are the only exceptions in which two-celled pollen grains are shed.

The pollen grains are acolpate and measure approximately 25 μ in diameter. The exine is marked with fine reticulations on the surface beset with minute spinescent projections. The intine appears as a thin and delicate membrane.

Ovule

There is only a single, laterally attached, bitegmic and crassinucellate ovule in each ovary. At first it is campylotropous but later becomes anatropous. At the megaspore mother cell stage both the integuments are already well advanced, each being two to three cells thick. Fig. 36 shows longitudinal section of an ovule at functioning megaspore stage. The inner integument alone forms the micropyle (Figs. 37, 38, 40). Its growth is rather slow in the beginning so that the nu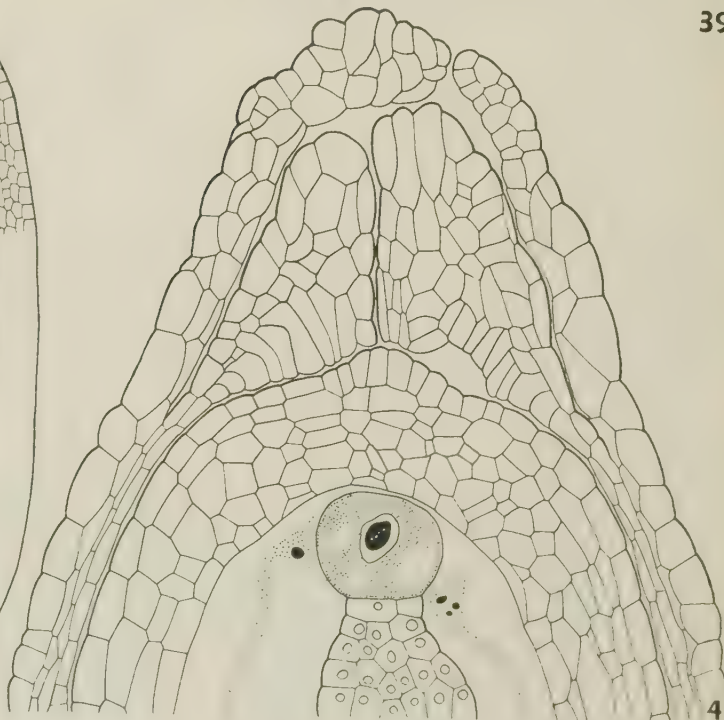cellus is not fully covered by it even at the eight-nucleate stage of the embryo sac (Fig. 39). However, by the time the polar nuclei have fused, it grows rapidly and its apical portion broadens to form the narrow micropyle (Figs. 37, 38). The

outer integument more or less covers the inner integument on the funicular side, but on the other side it is much shorter. After fertilization, however, the funicular part of the outer integument grows quickly and overarches the micropyle (Fig. 40).

Megasporogenesis

Several hypodermal archesporial cells may differentiate in the nucellus but only one of them functions as the megaspore mother cell after cutting off the primary parietal cell. Fig. 41 shows a multicelled archesporium while Fig. 42 shows a megaspore mother cell which has developed from a single-celled archesporium and in which the primary parietal cell has divided anticlinally to give rise to two daughter cells. Further divisions of the latter produce five to six layers of parietal tissue (Fig. 39). The megaspore mother cell enlarges considerably and crushes some of the adjoining cells. Campbell's (1898) statement that out of the two cells produced by the division of the hypodermal initial, it is the outer which functions as the real archesporium and divides to form the primary parietal cell and the sporogenous cell is incorrect.

The two dyad cells formed after the first division of the megaspore mother cell (Fig. 43) undergo another division and give rise to a tetrad of megaspores. Judging from the arrangement of the degenerated megaspores situated over the micropylar end of the embryo sac it may be concluded that the megaspores are arranged in a linear row (Figs. 45-47). Fig. 44 probably shows an oblique T-shaped tetrad in which one of the upper three megaspores has undergone a further division. The three degenerated megaspores persist for a long time and can be observed as deeply staining caps even in post-fertilization stages.

FIGS. 36-40 — Development of ovule. Figs. 36-38, l.s. ovules at functioning megaspore stage, mature embryo sac stage and just after fertilization; s = synergid, o = oospore and p.e. = primary endosperm nucleus. ×200. Figs. 39, 40, l.s. upper part of ovules showing condition of integuments and nucellus at mature embryo sac stage and after a young embryo has been formed. ×715, ×425.
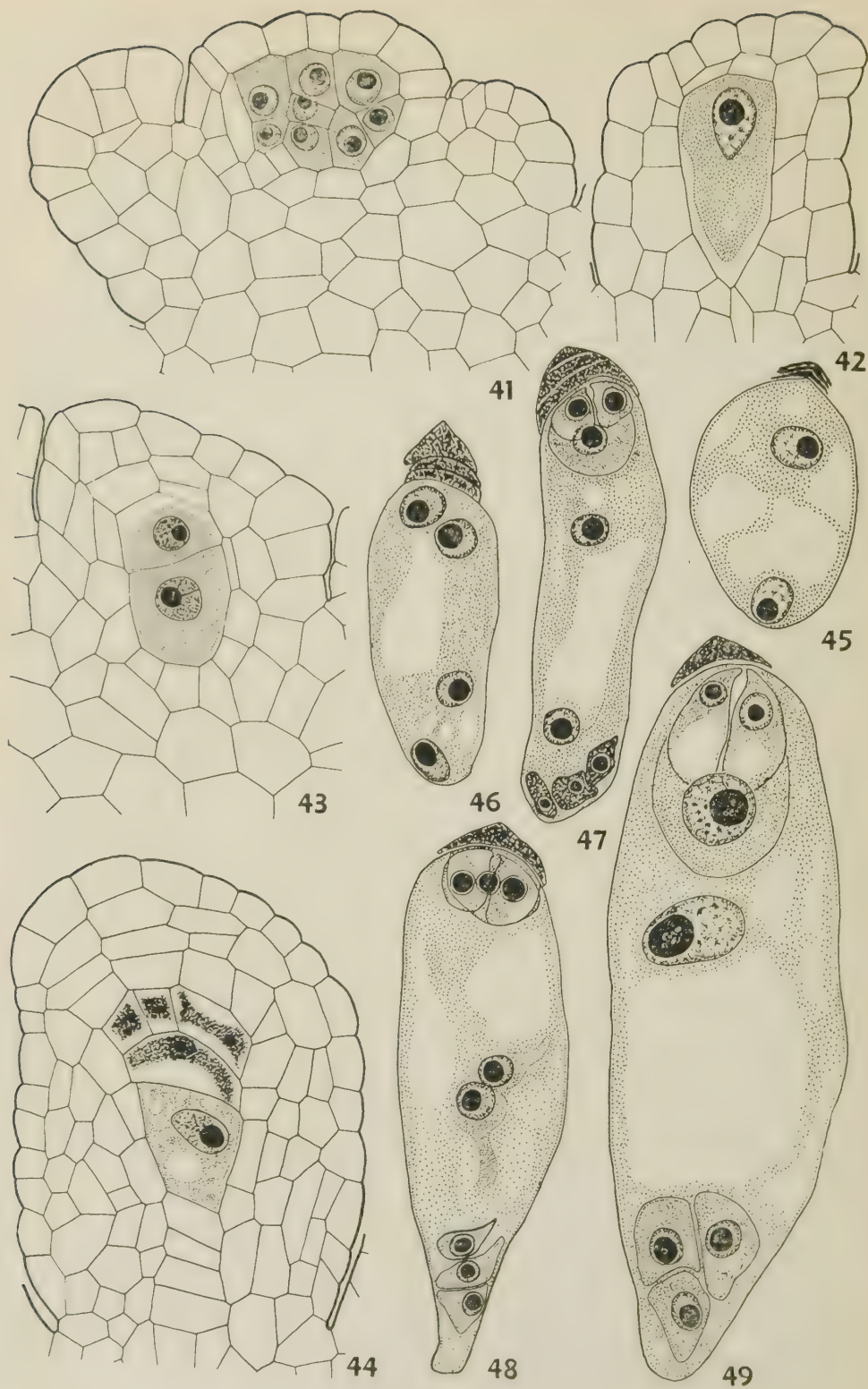
FIGS. 41-49

Campbell (1898) was unable to study the divisions of the megaspore mother cell, but says: " It is extremely unlikely that the primary sporogenous cell· ever develops at once into the embryo sac . . . In somewhat later stages there were found two or three cells derived from transverse divisions of the primary sporogenous cell, one of which by its subsequent growth destroys the others and becomes the embryo sac."

Embryo Sac

The lowest megaspore, which is the largest cell of the tetrad, enlarges further and its nucleus divides to produce two daughter nuclei which become separated by prominent vacuoles (Fig. 45). Both the nuclei divide twice giving rise to four- and eight-nucleate embryo sacs respectively (Figs. 46, 47).

From the micropylar group of four nuclei differentiate the egg apparatus consisting of two beaked synergids and the egg and the upper polar nucleus, while the chalazal group differentiates into the lower polar nucleus and three antipodal cells (Figs. 39, 47). The latter enlarge and become very prominent. During the formation of the embryo they shrink and degenerate but can still be observed as darkly stained masses at the base of the elongated chalazal end of the embryo sac (Fig. 57). Campbell (1898) states that the uppermost antipodal cell " projects strongly into the cavity of the embryo sac, and its nucleus becomes decidedly larger than those of the two lower cells ", but this could not be confirmed in my material.

The two polar nuclei fuse before fertilization almost in the middle of the embryo sac, which has considerably enlarged at this time measuring 20 µ broad at micropylar end and 62 µ long (Fig. 48). The

fusion nucleus moves to the upper part of the embryo sac and comes to lie just below the egg (Figs. 37, 49). Stenar (1935) reports that in *Scheuchzeria palustris* the fusion nucleus moves down to the chalazal end of the embryo sac.

Fertilization

Stages leading to the fertilization of the embryo sac have not been observed. In the fertilized embryo sac one synergid presents a disorganized appearance which is probably due to the impact of the pollen tube. The other synergid disorganizes soon after. The embryo sac, by this time, undergoes considerable elongation and is about four times as large as it was at the time of polar fusion (Fig. 48). Out of the large number of fertilized embryo sacs examined only one showed traces of the pollen tube. Evidently the pollen tube is a short-lived structure which is absorbed soon after it has entered the embryo sac and affected fertilization.

Endosperm and Embryo

The division of the primary endosperm nucleus always precedes the division of the oospore[6]. The earliest stage observed showed four free nuclei while the oospore was still undivided. (Fig. 50). Repeated divisions of the former produce many free nuclei arranged peripherally in a dense layer of cytoplasm (Fig. 51). Walls are not formed between the endosperm nuclei except near the basal cell of the embryo where a few endosperm cells are observed in late stages. With the growth and maturation of the embryo

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6. Hill's (1900) statement that in *Triglochin maritimum* the oospore divides earlier than the primary endosperm nucleus needs confirmation.

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FIGS. 41-49 — Megasporogenesis and development of the female gametophyte. Fig. 41, l.s. young. nucellus showing multi-celled archesporium. ×1100. Fig. 42, megaspore mother cell with primary parietal cell divided anticlinally. ×1100. Fig. 43, dyad cells. ×1100. Fig. 44, functioning megaspore (for further explanation see text). ×1100. Figs. 45-47, two-, four- and eight-nucleate embryo sacs. ×1100. Fig. 48, embryo sac showing polar nuclei lying adjacent to each other. ×1100. Fig. 49, same advanced stage showing fusion nucleus. ×1100.
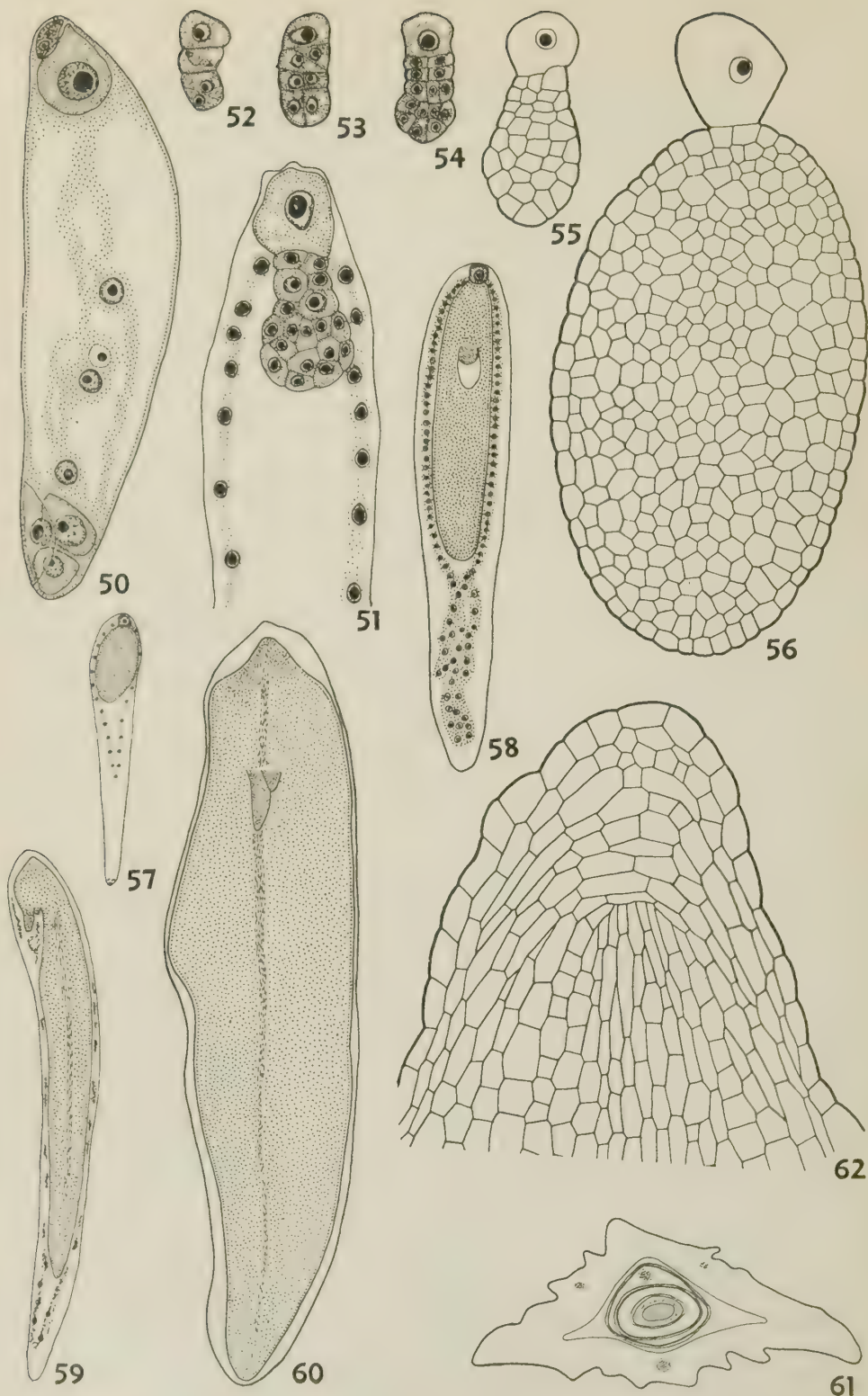
FIGS. 50-62
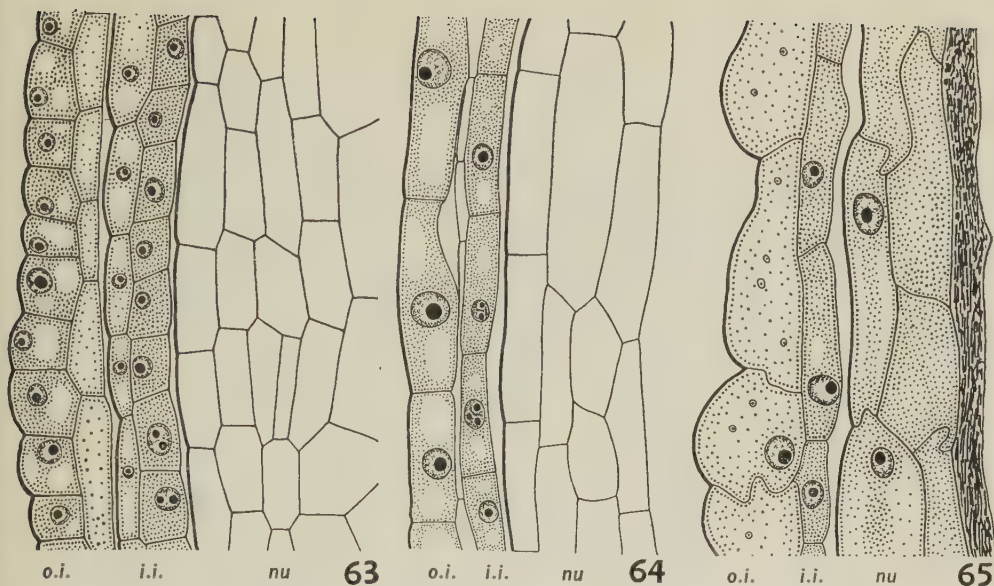
FIGS. 63-65 — Development of seed coat; stages showing changes in integuments during their transformation into the seed coat; *i.i.* = inner integument, *o.i.* = outer integument and *nu* = nucellus. ×715.

the endosperm nuclei are all crushed and absorbed (Figs. 59, 60) and the mature seed is non-endospermic (cf. Campbell, 1898). The embryo sac which has considerably elongated and now measures approximately 2800 μ is wholly occupied by the embryo (Fig. 60). Palm's (1915) inference that a helobial endosperm occurs in *Lilaea* is evidently incorrect.

My observations on the early development of the embryo, as shown in Figs. 52-56, are in general agreement with those of Campbell (1898). The first division of the oospore is transverse resulting in the formation of a basal cell and a terminal cell. The former enlarges considerably, does not divide any further

and persists for a long time so that it can be observed even in fairly advanced embryos (Fig. 58). A similar large undivided basal cell is reported in *Triglochin* (Hill, 1900; Souèges, 1943), *Scheuchzeria* (Stenar, 1935), *Naias* and *Lysichiton* (Campbell, 1897, 1900) and in most other members of the Helobiales (Maheshwari, 1950; p. 288). The terminal cell of the proembryo divides repeatedly producing a somewhat globular mass of cells (Figs. 56, 57) which continues to elongate (Figs. 58, 59) until it fills the entire embryo sac (Fig. 60). There is a single massive cotyledon whose cells are abundantly filled with starch grains (cf. Campbell, 1898). The lateral stem tip

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FIGS. 50-62 — Development of the endosperm and embryo. Fig. 50, embryo sac showing four free endosperm nuclei and undivided oospore. ×425. Fig. 51, upper part of embryo sac showing young embryo and peripherally arranged endosperm nuclei. ×425. Figs. 52-56, stages in the development of the young embryo. ×330. Fig. 57, embryo sac with the embryo shown in Fig. 56 surrounded by free endosperm nuclei; the degenerated antipodals are still persisting. ×42. Fig. 58, same, showing advanced embryo and endosperm. The basal cell is still visible and the stem tip has differentiated. ×42. Fig. 59, more advanced embryo with the lateral stem tip and terminal radicle; the endosperm is disorganizing. ×42. Fig. 60, embryo sac showing mature embryo; endosperm consumed. ×42. Fig. 61, t.s. young fruit. ×425. Fig. 62, l.s. radicle end of embryo. × 42.
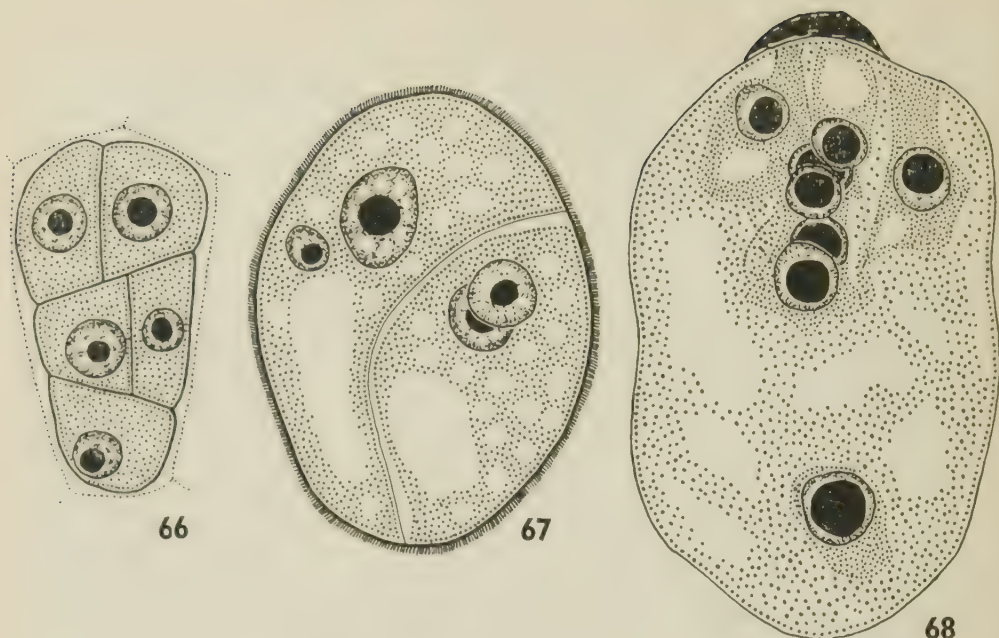
FIGS. 66-68 — Some abnormal stages. Fig. 66, five-celled tetrad of microspores. ×1650. Fig. 67, double pollen grain. ×1650. Fig. 68, embryo sac with two of the antipodal nuclei lying adjacent to egg apparatus. ×1650.

and the terminal radicle differentiate at a late stage (Figs. 58, 59). Fig. 61 shows a cross section of the young fruit showing the embryo, endosperm, integuments and pericarp.

Campbell (1898) states that the root tip of *Lilaea subulata* differs from that of other monocotyledons in being decidedly lateral in position rather than terminal. My observations show, however, that the root tip is terminal as in other plants and not lateral (Fig. 62). I am not aware if a lateral root tip has been reported in any other angiosperm up to this time.

Seed Coat

During the maturation of the seed coat the outer layer of the outer integument enlarges and becomes thinly cutinized, and starch grains appear in its cells. At the same time the inner layer of the inner integument, which becomes richly protoplasmic soon after the eight-nucleate embryo sac has organized, secretes a thick layer of cuticle separating it from

the nucellus (Fig. 63). The two intermediary layers of cells — one of each integument — get crushed (Fig. 64) so that the testa consists of only two layers of cells (Fig. 65) except at the micropylar end where the two integuments are still separately distinguishable. As the embryo enlarges some of the nucellar cells also get crushed and only its outer two to three layers remain intact (Fig. 65).

Abnormalities

One microspore tetrad showed five cells instead of four (Fig. 66). Such a condition may have arisen by a supernumerary division of one of the microspores or because of some abnormalities during the meiotic division. Maheshwari (1949) cites the occurrence of polyspory in several other plants, e.g. *Rosa, Atraphaxis* and *Coffea*. Shoemaker (1926) noted six cells in a variety of apple called " Stayman Winesap " and in *Fuchsia* Beer (1906) found six to ten cells arising from the microspore mother cell.

AUTHOR	JUNCAGINACEAE	SCHEUCHZERIACEAE	LILAEACEAE
Bentham and Hooker (1883)	1. Triglochin 2. Scheuchzeria 3. Tetroncium 4. Lilaea	×	×
Engler and Prantl (1889)	1. Tetroncium 2. Triglochin 3. Scheuchzeria 4. Lilaea	×	×
Hutchinson (1934)	1. Cycnogeton 2. Triglochin 3. Tetroncium 4. Maundia	Scheuchzeria	Lilaea
Wettstein (1935)	×	1. Scheuchzeria 2. Triglochin 3. Lilaea	×

Frequently double pollen grains were seen in which the two cells were more or less identical in shape and size and each had usual vegetative and generative cells (Fig. 67). Presumably these pollen grains originated by a non-separation of two cells of the microspore tetrad.

In one embryo sac two of the antipodal nuclei appeared to have moved to the micropylar part so that only one antipodal cell was left at the chalazal end (Fig. 68). Cases of a similar nature have also been recorded in a few other plants (see Maheshwari, 1948, 1950). In another embryo sac, one of the antipodals seemed to have travelled up to the centre so as to lie close to the polar nuclei. A third embryo sac showed twelve nuclei. This may be due to supernumerary divisions of some of the existing nuclei or to a fusion of two embryo sacs. Campbell (1898) also reported an abnormal embryo sac showing eight nuclei in its upper part with imperfect cell walls and without any trace of an egg apparatus, but the structure of the lower part of the sac was not clearly determined.

Systematic Position

It has already been mentioned on p. 15 that there is difference of opinion regarding the systematic position of *Lilaea subulata*. Bentham and Hooker (1883) and Engler and Prantl (1889) include it in the family Juncaginaceae (= Scheuchzeriaceae). On the other hand Hieronymus (1892), Schumann (1892) and Hutchinson (1934) place it in a separate family, Lilaeaceae. The above table summarizes most of the existing views on the subject.

The present work, however, shows that embryologically *Lilaea* is not so far removed from *Triglochin* as to justify its assignment to a separate family. In both (i) there is a single ovule in the ovary, (ii) the mature pollen grains are two-celled and (iii) the endosperm is free-nuclear. In *Scheuchzeria*, however, (i) there are two or more ovules in an ovary, (ii) the pollen grains are shed at the three-celled stage, and (iii) the endosperm is helobial (see Table on p. 28).

On embryological grounds, therefore, *Lilaea* is more closely related to *Triglochin* than to *Scheuchzeria*, and if *Triglochin* and *Scheuchzeria* are placed in the same family, there is no case for removing *Lilaea* to a separate family. Further studies of a comparative nature are needed on the morphology of the vegetative and reproductive organs of all the

SUMMARY OF WORK DONE ON THE EMBRYOLOGY OF SCHEUCHZERIA, TRIGLOCHIN AND LILAEA

GENUS AND NAMES OF INVESTIGATORS	ANDROECIUM	POLLEN GRAINS	GYNOECIUM	OVULE	ARCHESPORIUM	EMBRYO SAC	DOUBLE FERTILIZATION	ENDOSPERM	EMBRYO	SEED
Scheuchzeria Schnarf, 1931 Stenar, 1935	6, free, anthers basifixed, dehiscence longitudinal	3-celled	3-6, shortly united towards the base, stigma sessile, papillate	2 or more, basal, anatropous	1, hypodermal	Normal type (antipodals not seen)	Occurs	Helobial	Sagittaria type	1-2, non-endospermic, ellipsoid
Triglochin Hill, 1900 Schnarf, 1931 Wulff, 1939	6, epiphyllous, anthers subsessile, 2-celled, dehiscence longitudinal	2-celled	6, connate, stigma sessile, papillate	1, basal, anatropous	...	Normal type (antipodals 3-14)	Occurs	Free-nuclear	Sagittaria type, polyembryony reported in *T. palustre*	Erect, non-endospermic
Lilaea Campbell, 1898 Schnarf, 1931 Present work	1, subsessile, 2-celled, dehiscence longitudinal	2-celled	1, either short- or long-styled, stigma papillate	1, basal, anatropous	1, hypodermal	Normal type (antipodals persistent)	Occurs	Free-nuclear	Sagittaria type	Erect, non-endospermic

three genera before a definite decision can be arrived at[7].

Summary

1. In *Lilaea* the inflorescence is a pedunculate spike with spirally arranged pairs of male and female flowers terminated by a male flower. Two long-styled female flowers arise laterally at the base and are nearly six times as large as the other female flowers. The male flower consists of a single stamen subtended by a perianth lobe; the female flower consists of a single carpel with one ovule.

2. The anther wall comprises the epidermis, endothecium, two middle layers and tapetum. The latter gives rise to a periplasmodium which is gradually consumed during development of the pollen grain. The tetrads may be isobilateral, linear, T-shaped or irregular. The mature pollen grain is two-celled.

3. The ovule is bitegmic, crassinucellate and lateral. The micropyle is formed by the inner integument.

4. One to several hypodermal archesporial cells differentiate in the young nucellus. Only one of these functions as the megaspore mother cell after cutting off a parietal cell. A tetrad of megaspores is formed and the chalazal cell develops into an eight-nucleate embryo sac.

5. The primary endosperm nucleus divides earlier than the oospore and gives rise to a free-nuclear endosperm. As the embryo matures it is gradually absorbed so that the seed is non-endospermic.

6. The basal cell formed after the first division of the oospore does not undergo any further division. A suspensor is absent, and the embryo has a massive cotyledon, lateral stem tip and terminal radicle.

7. Double pollen grains, each with a vegetative and a generative cell, fre-

7. Markgraf (1936) also thinks that *Triglochin* and *Lilaea* are more closely allied to each other than to *Scheuchzeria*. In fact, he expresses the view that *Scheuchzeria* forms a sort of connecting link between the Helobiales and the Liliaceae and might well be placed in a family by itself.

quently occur in the same loculus as the normal pollen grain.

8. The available embryological data do not support the erection of a separate family, Lilaeaceae, as this would remove the genus *Lilaea* from the closely related genus *Triglochin*.

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MORPHOLOGY AND THE TAXONOMIST

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Morphological studies of higher plants have passed through stages of progressive development from the time of Linnaeus to the present. The stages are associated with increased specialization by the worker, more penetrating investigations, and an emphasis that has shifted from purely descriptive studies of the gross morphology of the plant (organography) to those of the comparative morphology of structures of allied or presumably allied taxa. The contemporary morphologist deals with the evolutionary genesis of structures and their internal components. Ontogenetic and phylogenetic studies contribute to this approach, and anatomy and cytology (*sensu latiore*) have become handmaidens to morphology.

Today the morphologist works toward the re-solution of problems of origin, evolution, and interrelationship of homologous structures and of the plants that bear them. In other words, he applies morphology to the study of extant or extinct populations. Now, as in the time of Linnaeus, the basic functions of taxonomy are nomenclature, classification, and identification. But in addition there has been an increasing emphasis on phylogeny and effort to make classifications more nearly phylogenetic. Taxonomy of this type is basically descriptive taxonomy, now often referred to as *alpha* taxonomy. It is a fundamental type of taxonomy that must be practised now and in the future by anyone inventorying the flora of floristically little known areas, particularly areas of the tropics and subtropics.

Floras of most temperate and especially boreal regions have been studied intensively for a century or more, whereas tropical and subtropical areas, which occupy more than half the earth's land surface, are for the most part much less known floristically. These less-known areas continue to yield species and genera hitherto unknown or undescribed, and most existing inventories of their floras are based on inadequate collections. The amount of energetic and exploratory taxonomic research now devoted to the floras of tropical Africa, Indo-China, Malaya, Indonesia, the tropical Americas and Mexico represents current studies by 100 or more full-time professional taxonomists. This situation should dispel notions held by many uninformed botanists that we know the world's flora, or that taxonomists spend much of their time " working over old hay ".

This alpha taxonomy must of necessity precede studies by the physiologist, pathologist, cytogeneticist, anatomist, morphologist and others — otherwise how can these investigators know the material with which they work ? On the other hand, it is only after results of studies by men of these allied interests are made available to the taxonomist that he is in a position to re-assess the relationships of minor taxa and begin to re-solve problems of phylogeny.

Alpha taxonomy must continue for plants of less-known areas. But by the synthesis of available botanical data there has been, for plants of the better-known areas (mostly of temperate and cold-temperate regions), a closer approach to *omega* taxonomy, in which the classification more closely approaches the phylogenetic. In this connection, taxonomists have placed considerable emphasis on karyogenetical data, recognizing the fundamental significance of heredity to the validity of characters and to phyletic relationships.

Contrasting with this recognition of the value of the karyogenetic data, the contemporary record reveals a relative minority of taxonomic publications utilizing data contributed by the morphologist and anatomist. Much has been written and classical examples abound to support the contribution of the geneticist and karyologist to the partial clarification of taxonomic problems. It is time that the taxonomist's attention be focused on the possibility of further clarifications by the

additional co-ordination of data from morphological and anatomical investigations. It is to be hoped that the day will soon come when all taxonomists think in terms of phylogeny. As pointed out by Eames (1929) and emphasized by Bailey (1949, 1951), phylogenetic arrangements must be based upon all available data. These data cover too broad a field to be obtained by any one worker, and the taxonomist must seek and utilize data available from the contributions of botanical colleagues.

In the last few decades morphology has contributed greatly to the taxonomy of major taxa. Comparative anatomy of reproductive and vegetative structures must be a major basis of any future system of classification. A few examples illustrate their significance.

Recent morphological studies of families of the so-called " Amentiferae " contribute to a clearer view of the phyletic positions of the families involved. Fisher (1928) held that the flowers of Salicaceae are seemingly simple by reduction: by this view the family cannot be primitive. Hallock (1930) and Hjelmquist (1948) agreed that the Garryaceae belong in an order by themselves allied closely to the Umbelliferae and Cornaceae. Abbe and Earle (1940) supported the view that the Leitneriaceae show closest relationships within the Rosales or Geraniales. Tippo (1938), Heimsch and Wetmore (1939), Heimsch (1942), and Manning (1938) concluded that the Juglandaceae are not primitive; and some of these workers believed them allied to the Anacardiaceae. Tippo (1938) held that the Betulaceae are derivatives of hamamelidaceous stocks, a view supported also by studies of their floral morphology and anatomy by Abbe (1935). Berridge (1914), and more recently Tippo (1938), support the derivation of the Fagaceae from tri-carpellate stocks related to present-day Hamamelidaceae. The only recent paper rejecting most of these conclusions (and in several instances without reference to them) was that by Hjelmquist (1948) wherein most of the well-known views of Engler and Wettstein were upheld.

The removal of *Euptelea* from the Trochodendraceae, and of *Illicium, Schi-zandra*, and *Tetracentron* from the Magnoliaceae, and the elevation of these as five unigeneric families, provide an example of close collaboration between taxonomists (Smith, 1945, 1946, 1947) and morphologists (Bailey, 1945; Bailey & Nast, 1945; Nast & Bailey, 1946).

Placental type and connation of perianth parts have been the principal basis for the usual placing of the Cactaceae in or near the Parietales. Evidence from many sources indicates that gamopetaly has developed independently in various major taxa. Evidence from the floral morphology and anatomy (Buxbaum, 1944, 1948; Chorinsky, 1931; Hallier, 1912), from embryology (Mauritzon, 1934) and from seed morphology (Martin, 1946), all support the view that the phyletic position of the Cactaceae is within or near the Centrospermae — an order which otherwise is polypetalous.

Another example of the current view that gamopetaly has arisen many times, and lacks the great phyletic significance accorded it by Engler and by Bessey, is to be found in the Cucurbitaceae. By Engler this family was placed in the Campanulales and by Bessey (together with the Begoniaceae) in his Loasales — an order considered by him to have been derived from rosaceous stocks. While there is need for broad comparative studies of floral morphology (including the embryology) within this family, existing evidence strongly favours the view that it is a terminal and advanced family of a polypetalous order.

Comparative morphology and anatomy have clarified our understanding of the relationships of many families, but at the same time morphologists occasionally express disappointment at not finding this information applied in new floras and manuals. They should remember that the taxonomist treats the plants in conformance with a given system of classification (as that by Engler, Bessey, or others) and ordinarily does not attempt to incorporate major phyletic changes. Any classification, to be workable and practical, must account for at least one or more divisions (or phyla). Most taxonomists recognize the inadequacies and faults of

every existing system, but continue to use one best suited to their needs.

In this connection it must be remembered that the Bessey system (currently receiving increasing recognition in the U.S.A.) is nearly fifty years old and reflects no modern findings and has never been revised since Bessey's last paper in 1915. Hutchinson (1926, 1934, 1948) failed to accompany his phyletic views with supporting evidence, ignoring contributory data from other facets of botany. The systems of Skottsberg (1940) and of Pulle (1950) took account of some morphological contributions, more especially European ones. Each deserves serious review, not so much for acceptance *in toto* as for taxonomic validity of realignments of many orders and families. The skeletal system proposed by Tippo (1942) is admirable so far as it goes, but cannot be adopted by the taxonomist until the families of higher plants have been fitted into it.

Since about 1910, no less than ten different systems of classification have been proposed to displace those of Bentham and Hooker and of Engler. None of these begins to answer the requirements of a truly phylogenetic system, and no system of the foreseeable future can attain such a goal. The task of assembling the available data contributing to our knowledge of interfamilial relationships is formidable, and that of integrating it to form a new and presumably better system of classification probably is beyond the grasp of any one person. Such a system is greatly needed, however, and it is time that a group of workers representing the different interests of botanical study undertake the project. With the appearance of such a system, accompanied as it must be by analyses of reasons underlying the phylogenetic and taxonomic position accorded each major taxon, the morphologist may expect to find his abundance of contributory evidence utilized, or at least recognized, by the taxonomist.

It is with the minor units of classification, especially genera and species, that the working taxonomist is most actively concerned. Perhaps no unit of classification offers more challenge in determining its circumscription and sometimes its

family position than does the genus. Two situations need consideration here: (1) the lack of clear-cut distinction between genera, and (2) the apparent distinctness of genera because it is not known if intermediates exist. Within many families, the accepted bases for separation of genera are weak; among such families are the Palmae, Amaryllidaceae, Aizoaceae, Cruciferae, Crassulaceae, Leguminosae, Cactaceae, Asclepiadaceae, and Bignoniaceae. When exomorphic characters have been found inadequate for establishing natural generic limits, the taxonomist needs other data. Often those of karyology and genetics fail to substantiate the taxonomists' long-established views (as in validity of genera of Orchidaceae), or the karyogenetic situations are so divergent within a genus as to suggest elevation of species or small groups of species to generic rank (as in some Leguminosae).

Papers have been presented by the morphologist and anatomist that clarify many of these problems and for others indicate more sound bases for realignment of genera within the family. Norris (1941) used vascular anatomy and nectary characteristics in an evaluation of some genera and the families of the Rhoeadales. His conclusions on the significance of nectaries were based on the association of them with their origin and vascular anatomy, not on mere external characteristics as used earlier by Hayek (1911). Corner (1946) pointed out the phyletic significance of centrifugal stamens, using this character as one additional reason for removing *Paeonia* from Ranunculaceae (whose other genera possess centripetal androecia), and concurring with Worsdell (1908) that the genus represents a unigeneric family, Paeoniaceae, allied to the Dilleniaceae. Lindsey (1938) presented evidence, based on floral anatomy, to support the view that *Menyanthes* deserved recognition as a family by itself, apart from the Gentianaceae in which it is conventionally placed. Morphological and karyological studies by Cave (1948) produced evidence rejecting the view that *Hemerocallis* belongs in the Amaryllidaceae (as currently held by Traub and associates) and

indicated that there is no close alliance to other members of the Hemerocallideae (Liliaceae). She suggested that *Leucocrinum* should be removed from that tribe and placed in a tribe by itself and " that embryologically *Hesperocallis* and *Hosta* are more closely similar to each other and to the genera in the Yucca-Agave group [Agavaceae of Hutchinson] than to . . . *Hemerocallis* and *Leucocrinum* ". Baumann (1946) published a detailed analysis of fruit morphology in the Umbelliferae, revised several earlier concepts of some intergeneric relationships and treated the tropical *Mydocarpus* as the most primitive of the genera. Kirchheimer (1948) presented new morphological evidence based on studies of carpels and locules for species phylogeny in *Cornus* and for generic relationships within the family. The study by Money, Bailey and Swamy (1950) on the Monimiaceae, and about 20 related families, is an excellent example of the synthesis of a wide range of data in the circumscription of genera and an alignment that emphasized the reticulate rather than the lineal phyletic pattern into which they appear to fit.

The contributions of the morphologist cited above (a few selections from a vast literature) are of a type to be utilized by the taxonomist dealing with regional and floristic studies. Contributions of this calibre have been appearing with increasing frequency for the last two or three decades. It has been suggested that the taxonomist is not sufficiently aware of contemporary literature in the fields of morphology and anatomy, and that too seldom is he cognizant of that appearing in foreign-language periodicals. It has been suggested also that the average taxonomist devotes his interests to plants of a particular and often narrowly circumscribed geographic area, making little pretence of knowing those of other regions or continents. There is basis for these suggestions, but they should not be accepted as statements of generalities. The taxonomist is expanding his horizons and is demonstrating to an increasing degree that his individual research reflects a synthesis of available data, and that he is bringing these data to bear on each problem in so far as they are available.

Interest in taxonomy is rising and with it the number of graduate students entering the field. Present-day graduate studies in taxonomy reflect a greater breadth of the subject than heretofore. Work produced in most centres of higher learning indicates clearly an appreciation of morphological and anatomical data as well as that of karyogenetics, and students are taught to search for and to utilize them. This revival of interest and expansion of scope of taxonomic work is encouraging. It is improving the attitude of botanists in general toward taxonomists in particular, and it points directly toward achievement of new and higher levels of taxonomic research. The taxonomist owes much to the morphologist and at the same time urges that more attention be given to the re-solution of some of the morphological aspects of taxonomic problems, especially as relate to generic delimitation and phyletic position. Like the taxonomist, the morphologist has restricted his studies to plants of temperate regions. The taxonomist desperately needs data from comparative morphological studies of many tropical and subtropical families. Some of these have been cited above; others include the Araceae, Proteaceae, the Gesneriaceae, and the Malvaceae-Bombacaceae-Sterculiaceae alliance.

The number of problems in this field is indeed great, and in their elucidation many taxonomists would gladly collaborate with the morphologist. There is opportunity here for a new era in co-operative research. It is time that each of us ceased to operate in self-made " cells " and to recognize the merit of " symbiosis " between fellow scientists. No morphologist or other botanist should think the progressive and alert taxonomist to be a complacent isolationist, to be a hibernating " herbariumist ", nor to be one oblivious of that which goes on about him in related fields of botanical endeavour. If publications of the last twenty-five years are a guide, it can be assumed safely that the day of that type of taxonomist is in a decline and that taxonomy, no longer a science dependent only on itself, is rapidly becoming one of synthesis.

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HETEROECISM IN *PUCCINIA INVENUSTA* SYD.

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Puccinia invenusta, first described by Sydow and Butler (1907), occurs in various parts of India on the leaves and rarely on the leaf sheaths of *Phragmites karka*, a common river-side plant. The writer (Sanwal, 1952) recorded its occurrence at Delhi a few months ago. Since certain other rusts on *Phragmites* have *Rumex* as their aecial host, it was considered probable that this rust may also be forming its aecia on some wild species of *Rumex* occurring in Delhi. However, laboratory infection of the leaves of *Rumex dentatus* by germinated teliospores was unsuccessful. In October 1951, the writer noted some infected leaves (aecial stage) of *Polygonum hydropiper* in the vicinity of certain plants of *Phragmites karka* bearing the uredial and telial stages of *Puccinia invenusta*. This occurrence of two stages in close association led him to suspect that a possible connection existed between the two rusts. This suspicion has been confirmed by inoculation studies, thus proving the heteroecism of *Puccinia invenusta*.

Materials and Methods

Material of the telial stage was collected in October 1951. It was immediately stored in paper bags and kept at room temperature. For observing cytological details, the teliospores were germinated on glass slides by the method suggested by Thirumalachar (1940). For carrying out infection tests the teliospores were floated on the surface of ordinary tap water in a petri dish and, when they had given rise to sporidia, a platinum loop was dipped in water and this was placed on the upper surface of the leaves of *Polygonum hydropiper*. Small portions of the trailing stems of this species were brought to the laboratory and placed on a thick wet pad of cotton inside a small jar under

aseptic conditions. Adventitious roots soon arise from the nodes and fix the plant to the cotton pad. At the same time the young leaves start unfolding. Only young leaves were used in inoculation experiments, although mature leaves offer no appreciable hindrance to sporidial infection. Inoculation of the old leaves of *Phragmites* by the aeciospores from *Polygonum* was done in large petri dishes by the floating leaf method suggested by Clinton and McCormick (1924). Direct inoculation of the leaves was also done at the original site of collection by the glass tube method used by Prasada (1947) for infection experiments with *Berberis*. Customary methods of fixing and embedding were followed for the observation of cytological and histological details.

Observations

UREDIA — The uredial and telial stage was described by Sydow and Butler (1907). At Delhi the uredia appear at the end of June. They are much elongated, brown, erumpent and pulverulent. The urediospores are yellowish brown, with an occasionally echinulate, uniformly thick wall (Fig. 1). They measure 16 μ to 26 μ. Four germ-pores are present, which become very distinct at the time of germination. The urediospores germinate readily on slides and produce a wide germ tube (Fig. 2). There is 100 per cent germination at room temperature (22°-30°C.), but the viability of the urediospores is lost in about a month's time in material stored in the laboratory. Secondary infection of *Phragmites* leaves takes place readily and the incubation period is about 15 days. Secondary spread, however, is of minor importance because sufficient aeciospores are available even up to February and the uredia
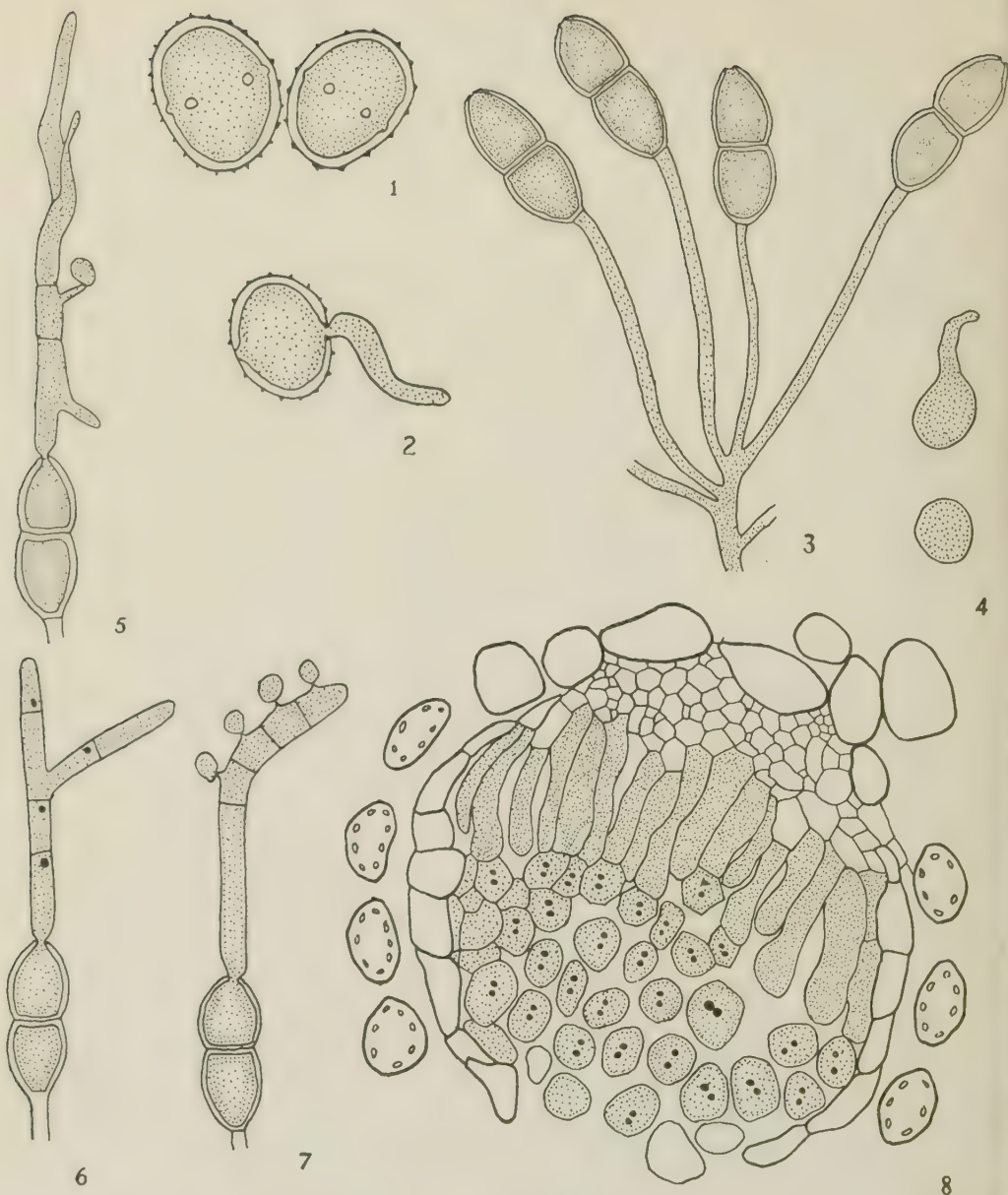
FIGS. 1-8 — Fig. 1, urediospores. ⁄720. Fig. 2, germinating urediospore. ⁄720. Fig. 3, teliospores arising from a common sporogenous basal cell. ⁄472. Fig. 4, sporidia, one germinating. ⁄720. Fig. 5, germinating teliospore showing the abnormal development of the sterigmata. ⁄472. Fig. 6, germinating teliospore showing the branching promycelium. ⁄472. Fig. 7, germinating teliospore showing the normal development of basidium and sporidia. ⁄472. Fig. 8, t.s. of the aecial cup (young). ×472.

readily give place to telia after a very brief period of production of urediospores. Additional grass hosts of *Puccinia invenusta* are not known at present.

TELIA — The telia start appearing amongst the uredial pustules towards the beginning of July. After this they are produced exclusively and uredia become very rare. The teliospores form a thick, elongated and velvety cushion on the upper surface of the leaf. Very rarely do they occur on the lower surface of the leaf.

The teliospores are dark brown, bi-celled, with a very long stalk. The measurements tally with those given by Sydow and Butler (1907). Many teliospores arise from a common basal cell (Fig. 3). These basal cells were overlooked by Sydow but were recently described by the writer (1952). As pointed out by Thirumalachar and Cummins (1949) these sporogenous basal cells have no taxonomic significance, having been observed in various related as well as unrelated species. The walls of the teliospores are very thick, with an apical pore in the upper and a lateral pore in the lower cell respectively. Mature teliospores possess a single nucleus.

The teliospores germinate readily upon maturity. Apparently no rest period is involved which is different from the condition in most species of *Puccinia*. On glass slides the germination is never more than 50 per cent, but on the surface of tap water it is as high as 80-90 per cent. Sporidia are formed within twelve hours. They are small, thin-walled, hyaline, rounded and are 5 μ in diameter. They start germinating by a slender germ tube *in situ* (Figs. 4, 7). Germination on the surface of water is mostly uniform and the teliospores germinate by means of a short promycelium, the apical part of which gets divided into four basidial cells, each of which produces a single sporidium on a short sterigma (Fig. 7). In slide cultures, however, certain abnormalities may occur (Figs. 5, 6). In some cases the promycelium may branch (Fig. 6). In others the sterigmata become very long and filamentous (Fig. 5). Such abnormalities have been seen in the germination of many rusts and are

usually ascribed to the presence of excessive moisture.

PYCNIA AND AECIA — The aecial stage on *Polygonum* was first reported from India by Sydow and Butler (1906) as *Aecidium polygoni-cuspidati* Dietel. This aecidium was originally described from Japan by Dietel (1903) on *Polygonum cuspidati*. In India it has been reported on *Polygonum hydropiper* and *P. glabrum* from Sylhet, Assam. The writer has not carried out cross inoculations on species of *Polygonum* other than *P. hydropiper*, so it is possible that the aecial stage of *Puccinia invenusta* also occurs on other species of this genus.

Young leaves of *Polygonum hydropiper* inoculated with sporidia in the laboratory on October 29, 1951, showed mature aecia on November 17, 1951, while the controls remained healthy under similar conditions. The first visible sign of infection on the leaves is the appearance of minute, yellowish discolourations on the upper surface of the leaves. Pycnia appear within seven days as greenish black, viscid specks. They are subepidermal, conical structures with many ostiolar paraphyses. The aecia, when mature, are cup-like and whitish, with dentate margins. They are invariably hypophyllous. A true peridium is lacking and in its place a pseudo-peridium is present (Fig. 8). The cells of the pseudo-peridium are thick-walled and polygonal without any markings on their walls. The hymenial cells are much longer than broad and have dense cytoplasmic contents (Fig. 8). The binucleate aeciospores become deranged in the aecium at a rather early stage and, therefore, show no trace of catenulation. Spore measurements agree with those given by Dietel for *Aecidium polygoni-cuspidati*.

Mature aeciospores germinate very readily on glass slides. When applied to the leaves of *Phragmites karka*, both in the laboratory and outside, they form typical uredia of *Puccinia invenusta* in about 20 days.

Discussion and Summary

The heteroecious nature of *Puccinia invenusta* was inferred by the observation that infected plants of *Polygonum* and

Phragmites, aecial and telial hosts respectively, occur in nature in close proximity. This inference was confirmed by actual inoculation and cross inoculation tests in the laboratory. *Aecidium polygoni-cuspidati* thus becomes the aecial stage of *Puccinia invenusta*. It is considered possible that hosts other than *Polygonum hydropiper*, on which *Aecidium polygoni-cuspidati* is recorded, may also serve as the aecial hosts of the rust under consideration.

The writer is indebted to Prof. P. Maheshwari for help and encouragement in many ways. He also takes this opportunity of thanking his colleague, Mr. S. K. Roy, for having taken great pains with the field work connected with this investigation.

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STUDIES IN THE BANGIOIDEAE
1. OBSERVATIONS ON *BANGIA FUSCOPURPUREA* (DILLW.) LYNGB. IN CULTURE

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Introduction

The observations here recorded form one section of a larger piece of work which has as its scope a closer understanding of the Bangioideae, the small and little-known subclass of the Rhodophyceae. Two of the genera, *Bangia* and *Porphyra*, are particularly common and certain species, such as the one to be considered, have extensive areas of distribution. *Bangia* is closely allied to *Porphyra*, but whereas the thallus of *Por-*phyra is a membranous expanse, collections of *Bangia* show a mixture of uniseriate and multiseriate filaments, the latter relatively complex. Several investigators have germinated spores of both these genera successfully in culture and recently Drew (1949) recorded the development of certain spores of *Porphyra umbilicalis* (L.) Kütz. into a shell-inhabiting organism, identical with growths described by Batters (1892) under the name of *Conchocelis rosea*. Spores such as these, which put out

narrow horizontally growing hypha-like filaments on germination, were said by previous workers to be carpospores, but this the present writer can neither confirm nor refute. The earlier workers also reported that, much less commonly, asexually formed spores are released from the thalli, and are to be distinguished by their completely different method of germination. These spores show bipolarity from the very beginning of development and the first wall in the already slightly elongated spore is parallel to the substratum. The lower cell becomes rhizoidal in shape and function and does not divide again, but the upper cell continues to divide to give a row of cells.

Whereas the " creeping filament " type of germination has been seen much more frequently than the " bi-polar " type in *Porphyra*, the reverse is true with regard to *Bangia*. Several workers[1] have watched the development of spores of *Bangia* into the beginnings of upright filaments but only Reinke (1878) and Kylin (1945) have reported seeing spores germinate into creeping filaments. Hence, when material of *Bangia fuscopurpurea* (Dillw.) Lyngb. became available at Rhosneigr, Anglesey, N. Wales, in December 1950, in a state of active reproduction, it seemed desirable to culture the spores particularly with a view to ascertaining whether *Bangia*, like *Porphyra*, has a shell-inhabiting phase. The spores which Reinke (1878) and later Kylin (1945) watched develop into creeping hypha-like filaments originated in both instances from material from Naples, during the winter months. During a short visit to the zoological station in Naples in April 1951, the opportunity of collecting material of *Bangia* from this locality was taken since it could be assumed that this material would be identical with that used by Reinke, Berthold and Kylin.

Some of this material was sent by air to Manchester and cultures obtained from it. In addition, material for general study was collected and fixed at Nervi, near Genoa, in February 1951 and at East Harbour, Alexandria, in March 1951.

Germlings of the " creeping filament " type have occurred in the cultures originating from material from Naples only. Unfortunately, they soon died and so whether they, like the spores of *Porphyra* which germinate in a similar manner, develop into a shell-inhabiting organism is still unknown. This account, therefore, is limited to observations on filaments resulting from the spores of material from both Wales and Naples, which show the " bi-polar " type of germination. These have been grown to maturity through several successive generations. Although grown under identical conditions, considerable differences have appeared between the cultures originating from the two localities, and the questions raised by these observations are subsequently discussed. The method of asexual reproduction shown by the Welsh material in culture points to the need for a fresh approach to the classification of the Bangiaceae.

Methods

The cultures, on which the observations were made, were obtained in the following manner. Filaments of *Bangia fuscopurpurea* from the shore were put on slides and flooded with culture solution. The slides were then put in a shallow covered dish in the bottom of which was thoroughly saturated filter paper to ensure a humid atmosphere. Spores were liberated during the subsequent days and these were then transferred to sterile culture dishes. Spores from material from N. Wales were put in the first instance on to sterile flakes of shell and sterile pieces of polyzoans and hydroids. All cultures except those on the shell flakes had to be discarded immediately owing to prolific growths of bacteria. Subsequent cultures have been grown on glass or Perspex. Subcultures have been made either by dropping sterile cover-

1. This type of germination was first recorded for the fresh-water *Bangia atropurpurea* (Roth.) Lyngb. by Derbes and Solier (1856) and this first account was followed by others by Cohn (1867), Goebel (1878), Berthold (1882) and Kylin (1922 and 1945) for marine material from various shores, named by all these investigators, *Bangia fuscopurpurea* (Dillw.) Lyngb.

lips or sterile pieces of Perspex into the parent culture for a short time or alternatively by lifting a single filament from the parent culture into a sterile petri dish.

Cultures have been grown in petri dishes of varying sizes and these have been kept on a glass tray touching the water surface of a water bath.[2] Fluorescent lamps suspended above the tank, giving a light intensity of approximately 3,500 lux when new, have been lit for twelve hours daily, but since the apparatus is against a west window, this artificial lighting has been supplemented by varying amounts and intensities of natural daylight.

The following culture solution has been used:

Filtered sea water from English Channel, three miles off shore from Plymouth		1 litre
Soil extract		50 cc.
Sodium nitrate	$NaNO_3$	0·1 gm.
Sodium phosphate	Na_2HPO_4	0·02 gm.
0·2% boric acid	H_3BO_3	0·5 cc.
0·1% manganese sulphate	$MnSO_4 4H_2O$	0·375 cc.
0·1% ferric citrate scales		0·625 cc.

The culture solution has been poured away from the culture dishes almost daily and not replaced until after a few hours, usually three to six, have elapsed. Cultures do not appear to suffer if left for an entire day without culture solution, neither do they appear to suffer if washed with distilled water.

Cultures of Bangia from Rhosneigr, Wales

Bangia fuscopurpurea occurs on steeply inclined rocks at high water level at various places along the shores of northwest Anglesey, North Wales, through the winter months. In 1950 it disappeared during the first weeks of June, but by mid-September a few very short filaments could be recognized with the aid of a

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2. No continuous records of temperature have been made but it is known to have ranged between 10° and 15°C. from December 1950 to April 1951 and between 15° and 20°C. from May to October 1951 inclusive. On a few summer days the temperature reached 22°C. for very short periods.

hand lens on a rock at Rhosneigr, where the species was abundant through the previous winter. Material consisting of uni- and multiseriate filaments was collected from this rock on December 11, 1950, and cultures were obtained from this material in the manner already described. Spores were liberated, apparently singly from cells of multiseriate filaments, and all germlings were of the upright or " bipolar " type (Fig. 2, K-N). In instances where spores germinated in the parent filaments, by no means a rare phenomenon, the rhizoid developed from the side of the spore towards the free surface of the filament. Within twenty-four days of spore liberation, the resulting filaments were a few millimetres long, were sporulating freely and short filaments of the next generation were already established. Between December 15, 1950 and October 29, 1951, sixteen generations have been grown to maturity and spore formation, although subculturing has not always been carried out at the first appearance of the spores. The onset of spore formation has usually occurred between two to three weeks after germination.

The constancy, rapidity and abundance of spore formation by methods to be described have been marked features of these cultures. The culture dishes have gradually become " colonized " by spores, many of which must incidentally be lost due to the practice of changing the culture solution frequently. Fig. 1, B, C, D, show the rate of " colonization " of a petri dish into which was put originally a single filament with mature spores, still distinguishable as a loop to the lower right-hand edge of the growth in Fig. 1, B.

In culture, the rosy-red filaments have not reached the length they do on the shore. The length attained in culture has varied somewhat, but the longer filaments have seldom exceeded 2 cm. and in other cultures they have never exceeded more than a few millimetres. The length to which the filaments attain appears to reflect the balance between the rate of cell division and the rate of spore formation and shedding from the end of the filament. One or other or possibly both of these processes appear to be
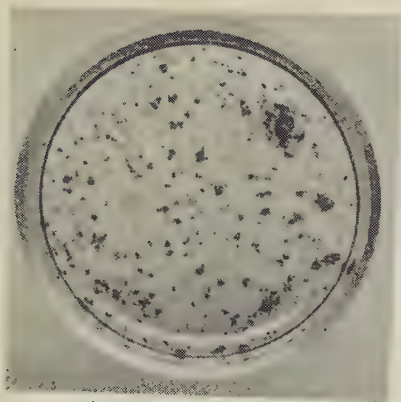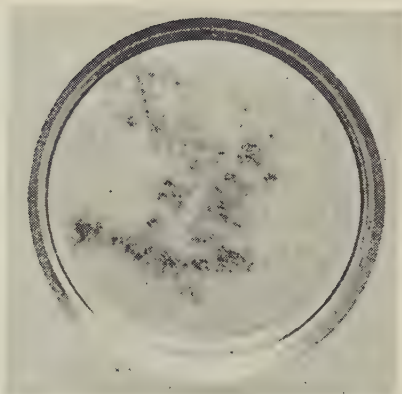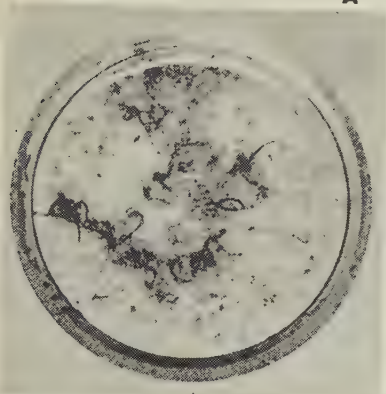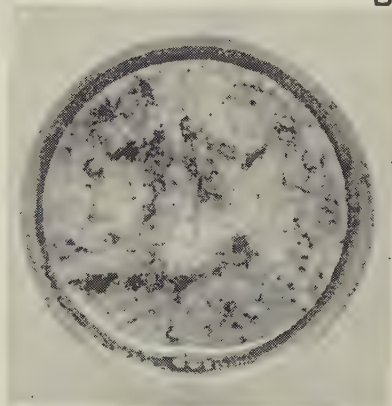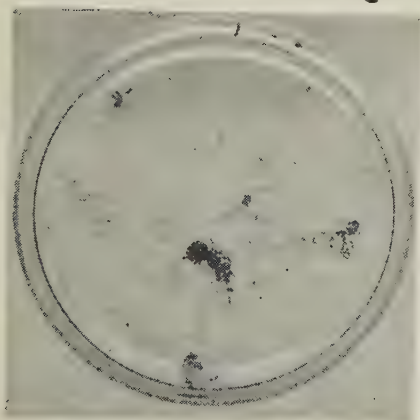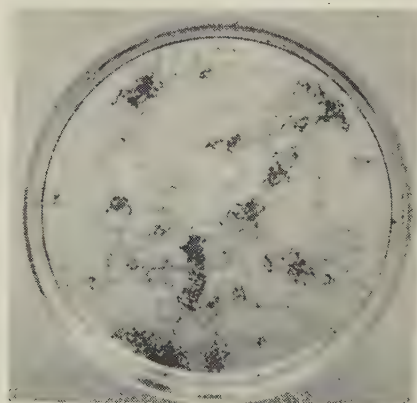
Fig. 1 — A, culture of Welsh material, 8th generation, 55 days old.   Photo : July 12, 1951.
B, culture of Welsh material, 13th generation, 31 days old.   Photo : August 27, 1951.   C, same
culture, 46 days old.   Photo : Sept. 11, 1951.   D, same culture, 61 days old. Photo : Sept. 26,
1951. E, culture of Naples material, 32 days old.   Photo : June 29, 1951.   F, same culture,
45 days old.   Photo : July 12, 1951.   All nat. size.

correlated with either temperature or
hours of daylight as it has been noticed
that the filaments have been very short
during the summer months. The tem-
perature and lighting of the culture tank
can be controlled within comparatively
wide limits only and so, as the year has
progressed, the average daily temperature
has risen and the daily artificial lighting
of twelve hours' duration has been sup-
plemented by more and more hours of
natural and brighter daylight. Fig. 1, A
shows a culture photographed on July
12, with extremely short filaments,
much shorter in fact than those of the
younger culture shown in Fig. 1 B grown
in a period of decreasing length of day.
In this connection it is of interest to
recall that Rosenvinge (1909) figures a
growth of very short filaments of *B.
fuscopurpurea* epiphytic on *Phyllitis zosteri-
folia* in August on the Danish coast.

Another noticeable feature of these
cultures is the absence of fully developed
multiseriate filaments and the prepon-
derance of uniseriate filaments or filaments
with few longitudinal or oblique divisions,
a situation found but infrequently on
the shore. After germination the young
filaments which show a characteristic cur-
vature from an early stage, increase in
length very quickly by means of inter-
calary growth, all cells except the basal
ones being able to divide (Fig. 2, A).
As they grow in length, the filaments also
increase in diameter (Fig. 2, B), young
uniseriate filaments having a diameter of
less than 15 µ but older ones reaching
45-50 µ. The filaments remain uniseriate
until after spore formation has started
at the apex, and many remain so indefi-
nitely. The cells of young filaments are
quadrate but those of older filaments
much shorter than broad. After the
onset of spore formation intercalary cell
division continues to take place, the cells
becoming shorter and shorter. Longi-
tudinal divisions may occur in the vege-
tative cells at any time from the on-
set of spore formation onwards and are
usually to be seen first at the apex
of the filaments, which have reached
a diameter of 25-40 µ (Fig. 2, B, C). A
longitudinal division of a quadrate cell
is often followed by a transverse division

in each daughter cell. In a few filaments
in older cultures, obliquely orientated
walls subdivide cells further (Fig. 2, D)
but this process is never carried far
and the widest filament measured was
65 µ in diameter. The filaments of
these cultures closely resemble those
figured by Kylin (1922). Printz (1926)
records a growth of *B. fuscopurpurea*
from Trondheim Fjord, consisting entirely
of narrow uniseriate filaments, 25-30 µ in
diameter, with a few longitudinal walls
at the apex of the filaments and thus
closely resembling those of the cultures
described. In two collections from N.
Wales and one from the Isle of Man,
made during early autumn months, fully
developed multiseriate filaments were
absent.

Within two to three weeks after ger-
mination, spore formation starts at the
apex of the filament and continues for a
considerable time. It starts by the
rounding up of the terminal cells of the
filament and the subsequent formation
of one spore from the entire contents of
each cell. Consequent on the breakdown
of the transverse walls and the partial
deliquescence of the outside walls, a row
of spores is liberated as shown in Fig. 2,
E, G, F and Fig. 4, A. Slight amoeboid
movement of these spores has been seen,
but if the filaments are left undisturbed,
the spores germinate quickly *in situ*.
Filaments in which longitudinal divisions
have taken place in the vegetative cells
give rise to two terminal ranks of spores
(Fig. 2, F). Germination of these spores
is always of the " bi-polar " type (Fig. 2,
K-N and Fig. 4, G).

While this type of spore formation is
responsible for the rapid " colonization "
of the culture dish in the first stages of
culture, intercalary spore formation has
been seen in cultures of a greater age.
In contrast to the method of spore for-
mation just described, several spores
develop in each cell concerned. Many of
the cells of such filaments are degenerate,
the reason for this not having been
ascertained although it appears to be
due to attack by a parasitic organism.
The cells which remain healthy, develop
thick walls, enlarge and in some ins-
tances divide vegetatively. The majority,
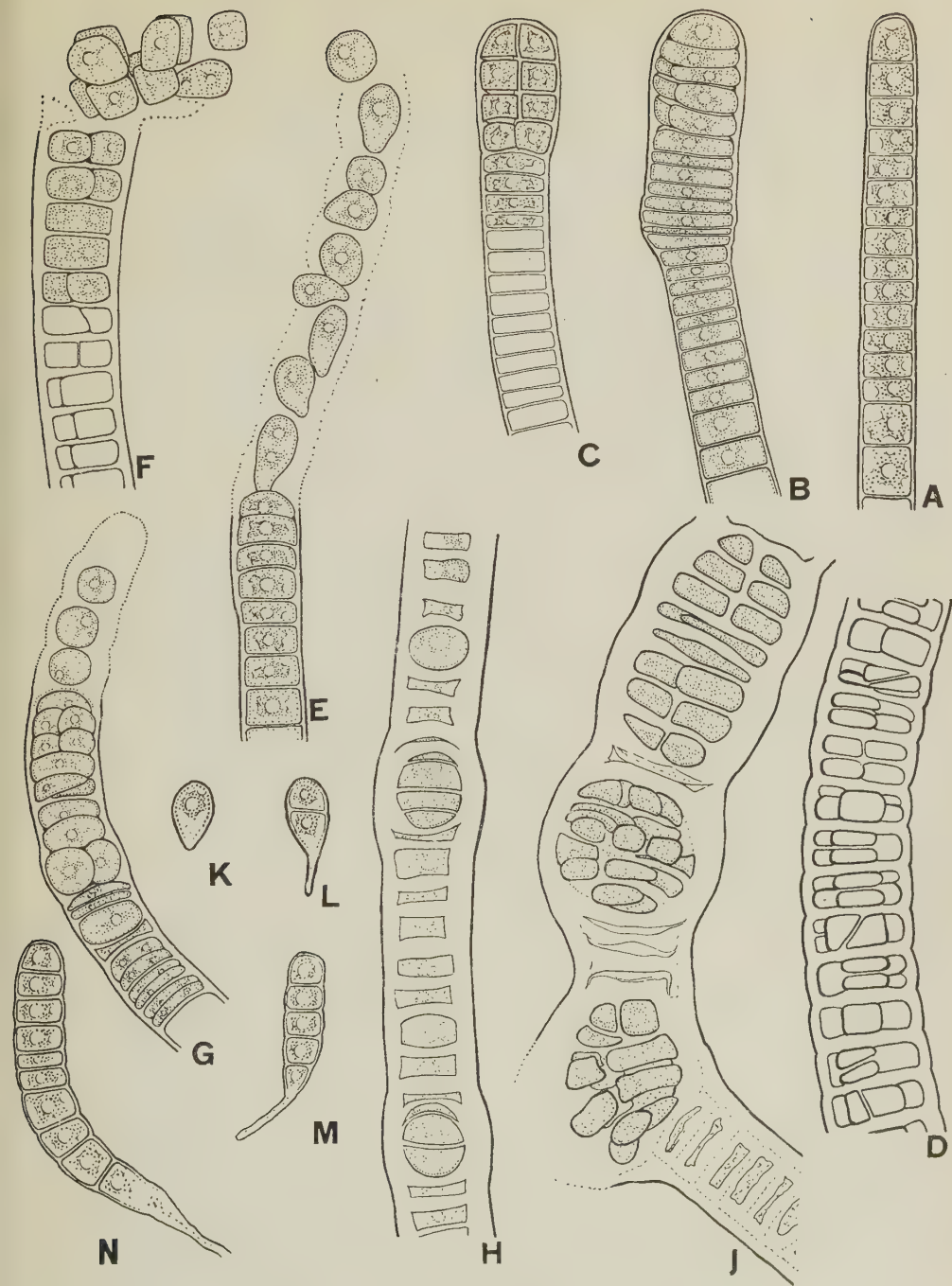
Fig. 2 — Cultures originating from material from Wales. A, young uniseriate filament. B, older filament showing increase in diameter and first longitudinal divisions. C, longitudinal division, associated with spore formation at apex of filament. D, typical example of most complex filaments developed. E-G, liberation of monospores from apex of filaments. Note some spores have started to divide. H-J, formation of intercalary sporangia. K-N, stages in germination of monospores. A, B, E, G, K, L, M, N drawn from living material. All. ×435.
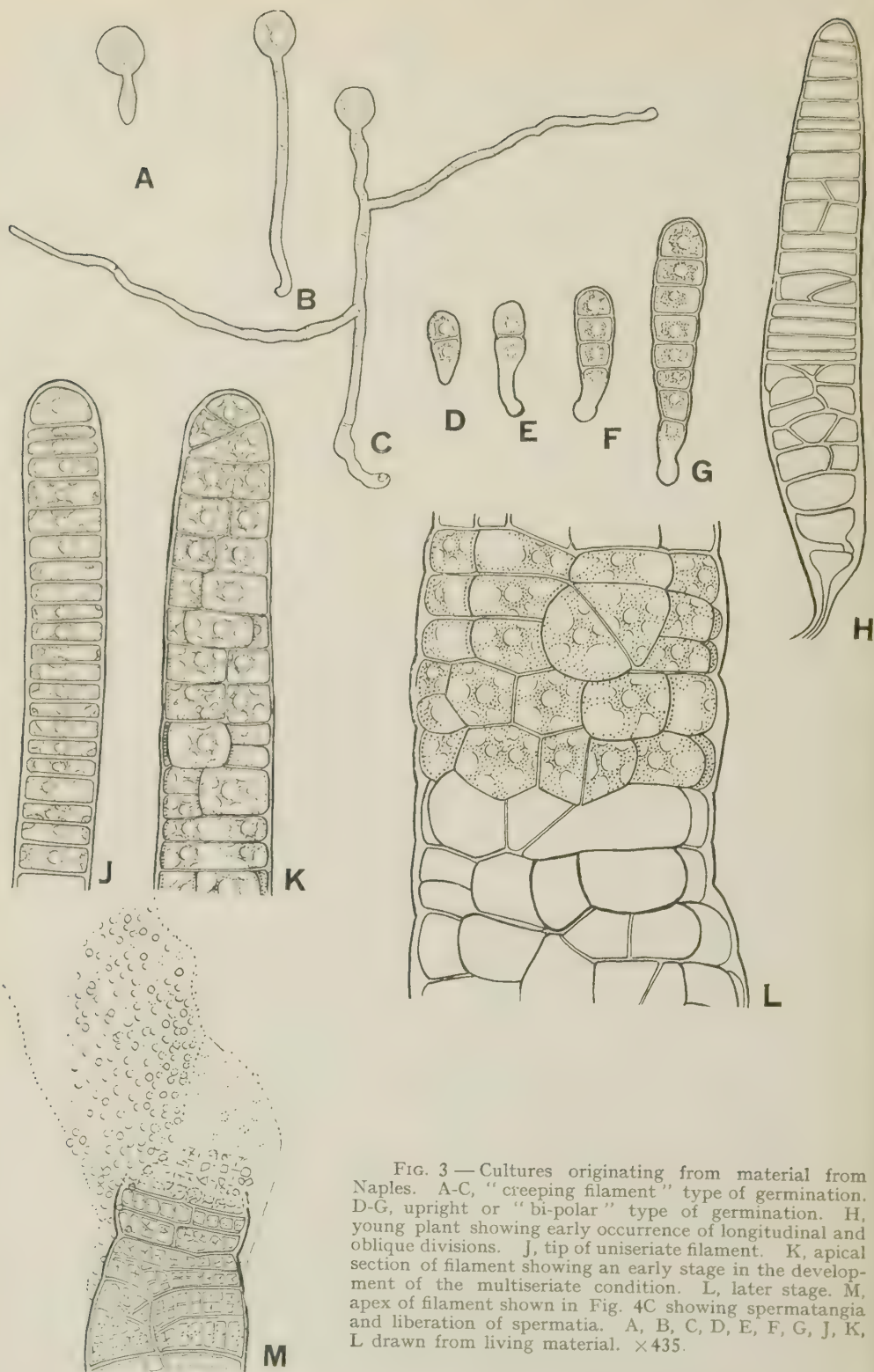
FIG. 3 — Cultures originating from material from Naples. A-C, "creeping filament" type of germination. D-G, upright or "bi-polar" type of germination. H, young plant showing early occurrence of longitudinal and oblique divisions. J, tip of uniseriate filament. K, apical section of filament showing an early stage in the development of the multiseriate condition. L, later stage. M, apex of filament shown in Fig. 4C showing spermatangia and liberation of spermatia. A, B, C, D, E, F, G, J, K, L drawn from living material. ×435.

however, divide up by transverse and longitudinal and even irregularly placed walls to give four, eight or often many spores, within the confines of the original more cell wall (Fig. 2, H, J). When two such filaments were transferred, each to separate sterile dishes, the spores were liberated rapidly and germinated very quickly. Germination was again of the " bi-polar " type.

Accounts of spore formation in *Bangia* in the existing literature leave much to be desired, but suggest that it is usual for more than one spore to be formed from each cell in multiseriate filaments. This has indeed been seen once, amongst material collected at Rhosneigr, N. Wales, in February 1951. The transformation of the entire contents of cells of uniseriate filaments into single spores, without mention of their position, is recorded by Kylin (1922) and Schmitz and Hauptfleisch (1896-97). However, there is no description in the literature clearly recording the method of spore formation and liberation from the apex of the filament, such as has occurred in these cultures. That this method is not the result of the conditions under which the plant has been grown, is shown by the fact that uniseriate filaments of a collection from the shore have behaved in a similar way when kept under observation in the laboratory.

The only record of intercalary spore formation with the formation of several spores per cell, which in any way resembles that which has occurred in the cultures, is given by Tanaka (1944) for *Bangia yamadai*, the figures of which (Fig. 7, *c*, *d*) show uniseriate filaments in each cell of which there are several spores. Tanaka labels these filaments as female but no positive evidence in favour of such a designation is given.

Sexual reproductive organs have not been seen in these cultures although filaments bearing spermatangia have been collected from the coast of Anglesey in both February and May of 1951. Dangeard (1927) found the sexual organs of *B. fuscopurpurea* near Quiberon in winter but quotes French writers as supporting the view that sexual reproductive organs are rare on the Atlantic and Channel coasts of France but common on the Mediterranean coast. The records in the literature are not sufficiently specific for a true estimate to be made of the frequency of sexual reproduction on the shores of the Atlantic but there seems to be reason for considering it infrequent on the shores of Sweden and Denmark.

Cultures of Material from Naples

Although the material used for this series of cultures was collected at Naples on April 14, 1951, it was the middle of May before spores from the filaments, which by that time were rather moribund, were germinated successfully. At first, the majority of the spores which germinated gave rise to creeping, horizontally growing filaments (Fig. 3, A-C), but gradually more of the germlings were of the " bi-polar " or upright type (Fig. 3, D-G). Unfortunately, the "creeping filament" type of germling, which resembled those described by Reinke (1878) and Kylin (1945) for *Bangia fuscopurpurea* and also those of *Porphyra umbilicalis*, from which the "*Conchocelis* phase" arises (Drew, 1949), gradually died after efforts to induce the filaments to penetrate shell flakes had failed. This can in no way be considered conclusive evidence that they never do so since the conditions were not sufficiently favourable when the attempt was made.

The germlings of the " bi-polar " type, however, remained healthy and have been grown to maturity and through a number of generations. The filaments in culture closely resembled those collected from the shore in every way, in contrast to the filaments of the Welsh cultures. At the same time the macroscopic appearance of the cultures of material originating from Naples differs markedly from those of material originating from Wales as a comparison of the culture dishes shown in Fig. 1, E, F and Fig. 1, A-D will show. The cultures of Fig. 1, B and Fig. 1, E are of about the same age and those of Fig. 1, C and Fig. 1, F. The filaments from Naples grow to a much greater length, sometimes reaching as much as 4 cm., and they are coarser than those from Wales. In
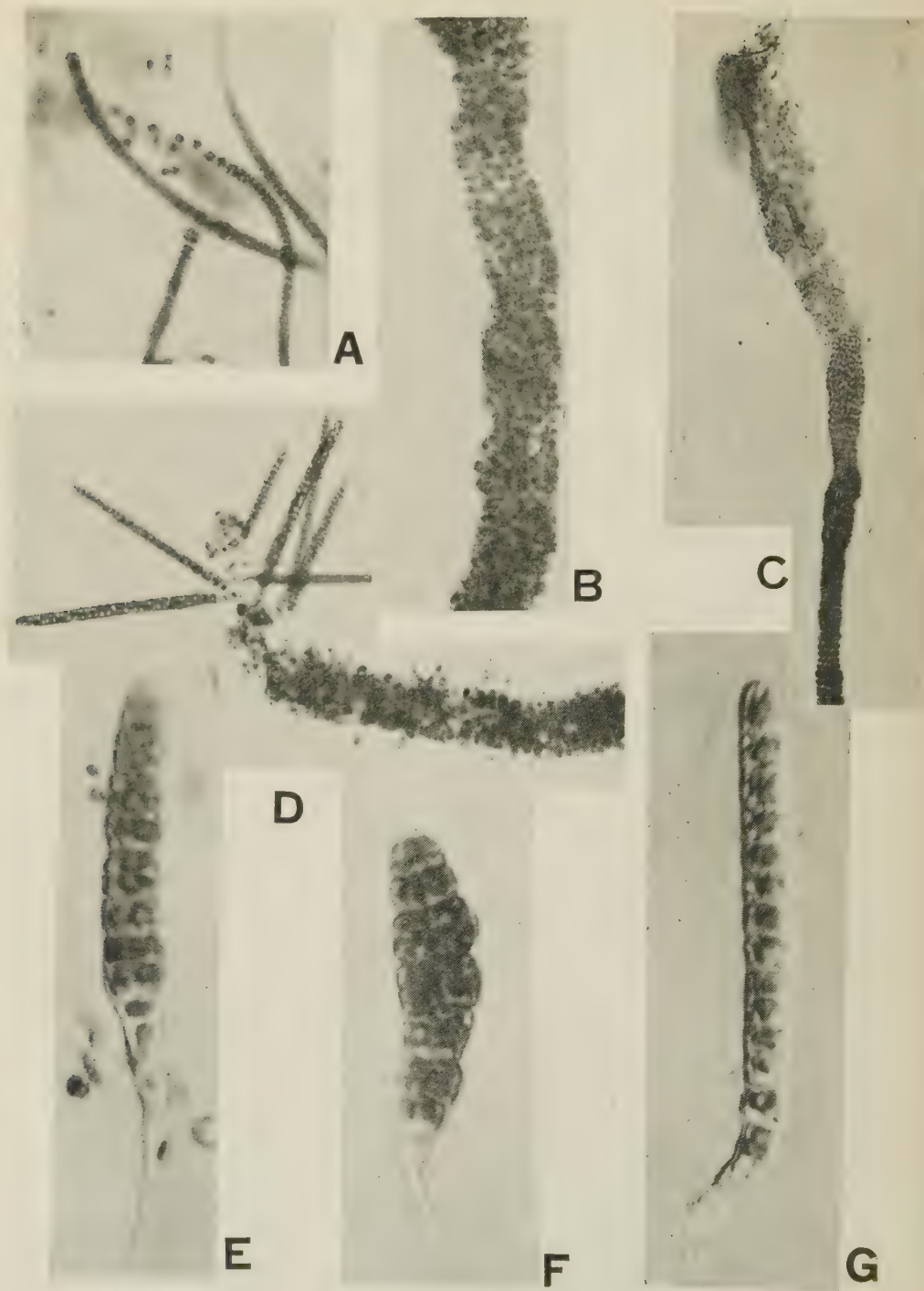
FIG. 4, A-G

addition, they are often twisted and curled and almost black in colour. Even in the earliest stages, these young filaments can be distinguished from corresponding filaments of the Welsh material by their slightly greater diameter and their darker colour as will be seen by a comparison of Fig. 4, E and G. While the majority of the young uniseriate filaments measured have been between 20 and 25 μ, a few were between 15 and 20 μ. As in the material from Wales, the first divisions in the already elongated spore differentiate a basal ˙rhizoidal attaching cell from an upper cell. The former does not divide again but the latter continues to divide transversely. Longitudinal divisions occasionally appear when the germling is only a few cells long (Fig. 3, H & Fig. 4, F), but it is more usual for these to appear when the filaments are a few millimetres long as Berthold (1882) recorded. By the time the filaments have reached this length, their diameter has increased to 30 or 35 μ and the rapidity of cell division results in the cells becoming very much broader than long (Fig. 3, J). Uniseriate filaments and filaments with only a few longitudinal divisions are very straight in contrast to the Welsh material, the filaments of which are characteristically arched. Most filaments with a diameter of between 30 and 50 μ show a certain number of longitudinal and oblique divisions (Fig. 3, K) except in the basal part of the filament, from the cells of which rhizoidal outgrowths develop. Filaments of over 50 μ in diameter have many complex divisions (Fig. 3, L & Fig. 4, B) and filaments up to 100 μ in diameter occur. There is no evidence to suggest that filaments remain in the uniseriate condition permanently. Longitudinal divisions may be initiated in various parts of the filament and, as cell division may proceed more rapidly on one side of a filament than the other, twisting and

contortion of the multiseriate filaments is almost universal. They thus contrast sharply with the very straight uniseriate filaments, a feature which can be seen with the aid of a lens in Fig. 1 E. Occasionally a filament appears to separate locally and one half grows more quickly than the other, giving rise to a loop.

Although the filaments of the Naples material grow to a much greater length and complexity than those of the Welsh material, the formation of spores is much less frequent and much more erratic, and so no regular subculturing of this material has been possible. The number of spores formed seems to be erratic also. Spore formation appears to be confined, possibly with very rare exceptions, to the multiseriate filaments, a single spore being formed from the contents of certain of the outermost cells (Fig. 4, D). Often this activity preponderates in the apex of the filament, but spore formation does not seem to be confined to that region. Since spores often germinate *in situ* it may happen that an old multiseriate filament may be crowned by a tuft of young uniseriate filaments (Fig. 4, D). It is interesting to note that this type of spore formation is similar to that in the material from Rhosneigr from which the cultures were started, and yet the resulting filaments show marked differences.

Spermatangia have developed in filaments in three culture dishes in relatively high numbers and spermatia have been discharged in masses starting at the apex of the filament in the way described by Dangeard (1927) (Fig. 3, M & Fig. 4, C). Carpogonia and carpospores have not been recognized.

Discussion

While no conclusions can be drawn from the observations recorded in the

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FIG. 4 — A, liberation of monospores from apex of living uniseriate filament. Wales culture. ×110. B, fully developed filament from Naples culture. ×110. C, filament liberating spermatia. Naples culture. ×110. D, liberation of monospores and germination *in situ* from multiseriate filament in Naples culture. ×110. E-F, young living plants from Naples culture. ×440. G, young living plant from Wales culture. ×440.

preceding pages — least of all regarding the life history of *Bangia fuscopurpurea* — the account given serves to point out the desirability of certain further investigations.

The most obvious of these is the need to enquire into the nature of the differences shown by the cultures to exist between the material from the two localities, Rhosneigr, N. Wales, and Naples. Firstly, although grown under identical conditions, from material which was sufficiently alike to be unhesitatingly classed as *Bangia fuscopurpurea* (Dillw.) Lyngb., cultures originating from material from Naples show differences from the earliest stages from those originating from material from Wales, and these become more marked as the cultures develop and age.

These differences can be summarized as follows:

1. The Naples cultures are almost black in colour whereas those from Wales are rosy red. The Naples cultures macroscopically resemble *Bangia* as collected on the shore and the filaments reach as much as 4 cm. in length. The Welsh cultures, on the other hand, consist of shorter and finer filaments than those usually occurring on the shore and seldom exceed a few millimetres in length.

2. Young uniseriate filaments from Naples are slightly broader than filaments of a corresponding age from Wales, the majority of the former being between 20 and 25 μ, whereas the latter are around 15 μ in diameter.

3. Filaments in the Naples cultures quickly become multiseriate (occasionally when only a few cells in length) and usually attain a state of maximum complexity such as occurs in the sea, but filaments in the Welsh cultures remain much shorter and often permanently uniseriate. Longitudinal divisions usually start in the filaments from Wales at the apical end of the filament and sometimes progress no further. They are never sufficiently numerous to produce filaments of any degree of complexity.

4. Spore formation is much less frequent and much more erratic in the Naples material than in the Welsh material.

5. Spores are liberated singly from the terminal cells of the uniseriate filaments of the Welsh material at an early stage, but as the filaments age, the cells in various parts of the filament appear to divide up within the confines of the original cell wall and liberate spores. Uniseriate filaments of the Naples material do not appear to liberate spores which are liberated singly from the outer cells of the multiseriate filaments only, particularly from the apical portion of the filaments.

6. The Naples filaments have formed spermatangia but no sexual cells have been seen in the Wales material.

These differences, which demand attention and investigation, may be found ultimately to have their explanation in one or more causes. The cultures of material originating from Naples and Wales may represent different phases of the life history. Although our knowledge of the life history is obviously incomplete, the evidence available suggests that identical phases have been cultured since both sets of cultures originated from similar filaments. There is no evidence in the literature for supposing that there is a separation of haploid sexual and diploid asexual phases in this species.

Another possibility is that the Naples and Welsh materials represent physiological races of one species and that the conditions under which the cultures have been grown are more nearly those obtaining at Naples than in N. Wales, permitting these filaments to attain the maximum complexity. This is certainly so as regards temperature, but this question could be answered only by culturing materials from both localities under a range of strictly controlled conditions.

This suggests that what we designate as *Bangia fuscopurpurea* is in reality an aggregate species. It is reported from a very wide area of the world, and when Laing (1928) noted its occurrence in New Zealand, he referred to it as an aggregate species. There have been attempts to separate it into various species, such as *B. crispa* Lyngb. and *B. pumila* Aresch., for example, but investigators who have

studied it extensively in restricted areas find such inter-grading that they include these forms under the one specific name (Levring, 1940). Kylin (1944) suggests that the differences on which such species were based are due to the influence of external factors, including seasonal changes. On morphological grounds alone, it should be noted, it is stated to be difficult to separate the fresh-water *B. atropurpurea* from the marine *B. fuscopurpurea*. An examination of material from N. Wales collected at various times of the year, together with the three isolated collections of fixed material from the Mediterranean (Genoa, Naples and Alexandria), showed a complete absence of morphological features, by which the Welsh and Mediterranean material could be distinguished. Thus the Welsh and Mediterranean material is phenotypically inseparable in nature, but under identical conditions of culture, genotypic differences become evident. In this connection it is worth noting that on the European seaboard of the Atlantic Ocean, *B. fuscopurpurea* is reported from about latitude 30°N. by Dangeard (1949) from the west coast of Morocco to Iceland, i.e. approximately the Arctic Circle (Jønsson, 1912). This is a phenomenally wide range. In the Mediterranean, the range is from just north of 30°N. at Alexandria to just north of 44°N. at Genoa. Incidentally this does not support the classification of *B. fuscopurpurea* as a boreal arctic species, as put forward by Jønsson (1912) and repeated by Levring (1940). Such a wide geographical range does suggest, however, that the differences seen in the cultures originating from Wales and Naples are expressions of genotypic differences of greater or lesser status, associated with geographical subdivisions of the great area inhabited by this species. Parallels among land plants are well known and so further investigations by means of cytology and culture of this species with this in mind are to be desired. Experiments in which land plants are transplanted from various parts of a wide geographical range and grown under identical conditions and investigated cytologically are well known, but they have yet to be undertaken for the algae, and this species is without

doubt a suitable one for such an investigation.

Should the material from the Atlantic come to be separated from that of the Mediterranean, then the name *B. fuscopurpurea* of Dillwyn (1809) will have to be kept for the material similar to that from which the cultures from Wales originated. This agrees closely as would be expected with that on which Dillwyn based the original description, since the latter was based on material collected by W. W. Young from limestone rocks a little above high water level in the neighbourhood of Dunraven Castle, Glamorganshire, S. Wales. It was described by Dillwyn (1809) in the following words : '' The filaments are quite simple, straight, rather entangled in their growth, and in length I believe seldom exceed an inch; when young their thickness is regular, but with age they swell so as in some places to be twice as thick as others. The dissepiments are so extremely slender that they can only be observed with the higher powers of the microscope. The joints are in length but about half equal to their thickness; they are nearly pellucid on each side towards the dissepiments and when the plant is old the juices collapse into globular granules, of which three are usually disposed transversely in each joint, though sometimes a single one occupies the whole.''

Without a close examination of *Bangia fuscopurpurea* from the southern part of the range in the Atlantic, it is impossible to do more than suggest that the form occurring there may be identical with the form inhabiting the Mediterranean. Should this be so, it is likely that at some places on the Atlantic seaboard, possibly the south coast of England, the areas of distribution of the two types or forms overlap.

The second point of interest to which these cultures have drawn attention is that they can be maintained more or less indefinitely by means of asexual reproduction. This fact is of importance for any consideration of the life history and ecology of *B. fuscopurpurea*, particularly the form on the coast of Wales. A survey of the available floristic and ecological records shows that *B. fuscopur-*

purea is absent, in the warmer latitudes at least, from its usual habitat, namely sharply inclined rock faces at high water level, during the warmest periods of the year. It is probable that further investigation will demonstrate the existence of a phase corresponding to the *Conchocelis* phase of *Porphyra umbilicalis*, but the cultures originating from the material from Wales have shown that this form of the species can exist as very short filaments, filaments which would be very inconspicuous on the shore or even if found in a sterile condition might easily be identified as a species of *Erythrotrichia*. The method of spore formation would exclude it from this genus, but as the terminal spores are quickly washed away by movement, it is doubtful whether they would be noticed readily on material taken straight from the sea. The cultures have shown also that the Welsh plant can live and reproduce abundantly under conditions which are very different from those which are recognized as its natural one, but conditions which could be expected at other places on shores and from which the plant could spread back to the rocks at high water level in the autumn, when conditions are suitable for growth there. This may, in fact, be one method, although not the only one, by which this species survives the period during which conditions are unsuitable for its growth at high water level.

As has been stated, the filaments in the cultures originating from material from Wales lack any features by which they could be distinguished in the sterile condition from species of *Erythrotrichia*. In addition, the method of liberation of a succession of monospores from the apical end of uniseriate or more rarely biseriate filaments is very similar to that described and figured by Smith (1943) for *Goniotrichum elegans*. Although the details given are not sufficient, there appears to be considerable similarity also to *Erythrotrichia biseriata* of Tanaka (1944). This suggests that the existing generic distinctions are inadequate, a situation already noted by other writers. For example, Dangeard (1949) questions whether *Erythrotrichia welwitschi* (Rupr.)

Batt. should not be included in the genus *Bangia*, and Smith (1943) in considering *Erythrotrichia pulvinata* suggests that in the absence of knowledge regarding spore formation, this plant might be justifiably considered a species of *Porphyra*.

Finally, the ease with which the Welsh material can be cultured suggests the use of this alga for other types of experimental investigation requiring a constant supply of living material. The correlations of growth and spore formation with external factors are obvious lines of enquiry.

Summary

Observations on cultures of *Bangia fuscopurpurea* originating from Wales and from Naples have confirmed the earlier accounts of the germination of the spores of this species.

In the case of the Wales material, sixteen generations have been grown to maturity. Reproduction in all cultures has been by means of asexual spores, but in addition spermatangia have developed in cultures originating from material from Naples.

Although kept under identical conditions, marked differences have existed between the cultures originating from Wales from those originating from Naples. This indicates the need for further investigation to determine whether *B. fuscopurpurea* is an aggregate species comprising physiological and morphological races.

The cultures of the Welsh material suggest that this form, which is probably identical with the type material, can exist in a simple form, which is of importance for the species biologically.

The methods of spore formation observed in these cultures suggest the need for a reconsideration of this process in the genus *Bangia* as well as an enquiry into the desirability of using methods of spore formation as a basis of generic distinction in this group of algae.

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BUD AUTOGAMY IN SOME NORTHERN ORCHIDS

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Introduction

The fantastic wealth of orchids in the tropics has an overpowering effect on the biologist who is walking for the first time beneath the palms, because each trunk of the primaeval forest seems to bring new and alluring puzzles both of a taxonomic and a morphological nature.

Behind a multiplicity of colours there are also hidden corresponding features of floral biology, e.g. pollination by a host of types of jungle insects which are particularly adapted for this purpose. But the number of observations made in nature on the aspect is still very small.

With increasing distance from the equator the number of species of orchids decreases rapidly, and relatively few are left in northern countries. Denmark possesses no more than 40 species, the majority of which are either uncommon or rare. In the Faroes (610) there are just 6, and only one of these is common. The northern boundary is a little north of the polar circle, and only a few small-flowered species reach close to the northern point

of Scandinavia. In the bleak polar climate of West Greenland the northernmost occurrence of an orchid is at Disko (about 70°N.) where it can thrive only near geysers.

From a biological point of view our native orchids may be roughly divided into small-flowered and relatively large-flowered species. Some of the large-flowered ones are pollinated by insects, but in the small-flowered ones the pollinating agency is more or less unknown.

In the tropics also there are many small-flowered species, the pollination of which needs to be investigated. Among these may be mentioned *Spiranthes australis*, in which fructification is so plentiful as to suggest autogamy. Almost every flower bears fruit (Maheshwari & Narayanaswami, 1952). The same thing appears to be true of some of the largest-flowered species like *Phajus wallichii* Lindl. and *Spathoglottis plicata* Bl., which are very common ornamental plants in Sumatra. Both of these species are autogamous. Kirchner (1922 a, b) has reviewed our knowledge of autogamy in tropical orchids.

With an increasing distance from the equator the number of species diminishes but there is no simplification of the floral biology. Significant examples are afforded by species of the genus *Ophrys*.

Orchis maculata is the northernmost, large-flowered, entomophilous orchid, occurring in the Atlantic area; its abundance at the Faroes (and in Iceland) is conditioned by the presence of a large fly (*Eristalis intricarius*), which acts as the pollinator (Hagerup, 1951).

To the north of the northern boundary of this autogamous plant, there are some small-flowered orchids whose ability to live in such climate is in no small measure due to their capability for pollination unassisted by insects. In certain places, e.g. the Faroes, insects are so few that they cannot play any significant rôle in pollination and the consequent formation of seeds. Nevertheless, the small-flowered orchids of the northernmost regions bear remarkably abundant fruit, producing large quantities of seeds. This is true, for example, of the two Greenland species of *Habenaria* (=*Gymnadenia, Leucorchis*)

which I also have had the opportunity of observing in other northern parts: Iceland, the Faroes and Denmark.

Regarding the northernmost orchids it may be noted that, besides being small, the flowers are also completely or partly closed, a phenomenon which might suggest autogamy in spite of the hints of Knuth (1899) and other writers about entomogamy. This also applies to *Spiranthes spiralis*, which flowers so late (October) in our regions that the humble-bees, which are said to act as pollinators, have already finished their season of activity.

For most of the existing observations on pollination in orchids we are indebted to some prominent flower biologists of the last century. They were, however, handicapped by the crude technique of those days which made it impossible for them to clarify the complicated structure and function of the small-flowered species. These technical difficulties were, no doubt, responsible for their poor understanding of the flower of *Herminium*, about which more will be mentioned in a later work. In fact, this is true of our knowledge of the pollination mechanism of the majority of the small-flowered orchids.

The technical shortcomings mentioned above can now be remedied by studying the flowers with the aid of serial microtome sections, which reveal many fine and interesting features of the morphology and biology of these flowers which older biologists were unable to study.

I collected much of the material for my investigations during various travels in Greenland, Iceland and Denmark. More profitable still were the various stays at the Faroes in 1922-23 and 1947. Especially at the Faroes there was, as far as I could see, no possibility for visits of insects to *Leucorchis albida*. The same holds good for the northernmost localities of this species in Greenland.

Later, my material was supplemented by the collections of others, preserved in spirit at the Botanical Museum of the University of Copenhagen.

None of the species mentioned below have proved to be apogamous, for in all of them the pollen grains were found to send pollen tubes down to the ovules.

Observations

LEUCORCHIS (GYMNADENIA) ALBIDA L.— The white, fragrant flower of this species has been examined several times by Müller (1881), Knuth (1899), and Ziegenspeck (1934), all of them stating that it is pollinated by butterflies. This may perhaps be the case in places like the Alps where insects are plentiful. Flowers have frequently been found there from which one or both pollinia had been removed.

Nevertheless, I am growing more and more suspicious that the flower might be autogamous because of the very abundant fructification of the plant even at such places in arctic regions where insects are too few to play a decisive rôle in pollination. My scepticism is supported by the fact that the flower is often partly closed and is oriented more or less horizontally (Fig. 1).

If a flower bud is dissected immediately before its emergence, the anther is found to have opened already by a slit along its inside (Fig. 3), and the massulae lie loosely inside it (Fig. 2) so that some of them easily fall out if the anther is gently touched, as in the process of placing it under the microscope.

A similar thing, no doubt, happens in nature, where, as the result of the shaking the plant receives by wind, some of the massulae fall out into the interior of the flower. However, even in the open flower many massulae are still left in the anther and may be transported to some distance by insects.

As in *Orchis,* all of the massulae are normally connected together by means of some cellular tissue lying between them. This tissue is, however, partially dissolved in the autogamous species described here.

Since the rostellum is quite short and narrow, it is no hindrance to autogamy and to the movements of the massulae.

When the flower bud has opened (Fig. 2), some of the massulae have already spread so effectively inside the flower that a few of them have even reached out between the perianth lobes. However, the majority fall directly below the anther thereby hitting the remarkably long styles which are papillate at the base. Their tips project beyond the entrance of the spur in such a position that they would be easily touched by the proboscis of an insect seeking honey.

HABENARIA HYPERBOREA L. — This little small-flowered orchid is very much like the preceding species both in appearance and in the mechanism of its pollination. The anther opens before the flower, and massulae are already present on the stigmas, where they immediately germinate. Unlike *H. albida* the rostellum also bears stigmatic papillae quite up to the tip.

In Greenland the species is found north of the polar circle together with the preceding species, both bearing abundant fruit in spite of the fact that there are few or no insect visits. The two species can scarcely be self-sterile, for their own pollen easily germinates on the stigma. Insect visits would probably be of little use to the plant, since the flowers have already pollinated themselves before opening (Fig. 4).

The inner surface of the spur is densely provided with glandular hairs secreting nectar, which can, however, be of little value in ordinary pollination.

COELOGLOSSUM (HABENARIA) VIRIDE L. (Figs. 5, 6) — A careful examination of this curious, half-closed flower was made long ago by Darwin (1904). and Müller (1881). There is in addition the rather unique phenomenon that here the entrance to the spur is closed by a thin membrane (Hagerup, 1951) which can be easily penetrated only by insects with strong, biting mandibles but scarcely by butterflies and rather small diptera.

In accordance with this, Silén (1906 a, p. 98) made the observation that in the long, lighted nights of Finland the flowers are visited by certain beetles (*Cantharis*), which transport the pollinia from flower to flower.

In the Faroes, where only a few pollinating insects exist, many flowers are nevertheless pollinated. The membrane had been burst and pollen was found on the stigma. In some of the pollinated flowers their own pollinia were still found to be left behind. However, among the fertile flowers there were some that

did not develop fruit, which is quite characteristic of such orchids which are entomophilous.

There is, therefore, hardly reason to doubt that *Coeloglossum* can be pollinated by insects. However, I am not sure as to how widespread this type of pollination is in nature. The uncertainty in my mind is due to the fact that even before the expansion of the flower the anther is wide open and shows a broad slit along its inner side. The individual massulae do not all cohere, but lie so loosely inside the anther that they easily fall out when shaken by the wind, etc. And even if an insect were strong enough to perforate the membrane and pull out the pollinia from the anther, it is almost inevitable that some of the massulae would fall down into the interior of the flower so that it is pollinated by its own pollen; although at the same time it may possibly receive some pollen from other flowers. It is not known whether the flower is self-sterile.

Accordingly, there seems to be considerable chance of autogamy; and this might occur not only before the visit of insects, but also later when the flower is open. Such a chance of autogamy is of much biological value in regions which are poor in insects, so that the species does not become extinct owing to paucity of pollination.

An Icelandic plant growing in the Botanical Garden, Copenhagen, was covered with a bag, but no fruits were set since it was not a self-pollinator.

GOODYERA REPENS L. — The structure of the flower has been examined several times by Darwin (1904), Müller (1881), and Ziegenspeck (1934); but the mechanism of pollination was not completely clarified. Most writers hold that the flower is entomophilous, since it possesses odour as well as honey and is visited by insects. In the Danish flowers examined by me, both pollen and the viscid gland had been removed, from which there is hardly any reason to doubt the visits of insects (*Bombus*).

In order to determine whether the flowers can also pollinate themselves, serial microtome sections were prepared of large buds fixed shortly before the opening. Fig. 8 shows an almost median, longitudinal section through such a bud. It is seen that the rostellum *E* (dotted) ends in a large viscid disk *F* (black) surrounded by mucilage, the back being covered with massulae which are free, independent of one another, and on the point of being liberated from the anther *D*. Some of the massulae have already landed at various places in the interior of the flower.

A few massulae have fallen downwards, having passed along the edges of the rostellum, but later they were held up in a viscid substance, secreted from the stigma which is situated immediately below the rostellum.

Thus *Goodyera*, too, like the preceding species, can pollinate itself and also receive pollen from other flowers. If it is not self-sterile, it is presumably its own pollen which will effect fertilization, as this will be the first to reach the stigma.

SPIRANTHES SPIRALIS L. (Figs. 9-12) — The flower was examined by Darwin (1904) and several later investigators who agree that it is pollinated by insects (humble-bees). But in Denmark, where the species is near its northern boundary, the activities of the humble-bees are already over at the time when the plant is in bloom; accordingly, the value of insect pollination needs to be re-tested. As the flower is almost closed and fructification is abundant, it is natural to suggest autogamy.

In *S. spiralis* many pollen-tubes are seen to penetrate inside the ovary and this species does not appear to be apogamous. Its tropical relative, *S. australis*, studied by Maheshwari and Narayanswami (1952), shows normal fertilization as well as apogamy.

To the present observations on the structure of the flower there may be added some others based on serial microtome sections.

The entrance to the short spur, shaped like a bag, is closed by two flaps *C* from the base of the labellum *D* (Fig. 12) which are very much like two folding doors (Fig. 11), at first lying almost at a right angle to the labellum but later bent down into the spur. They are densely covered
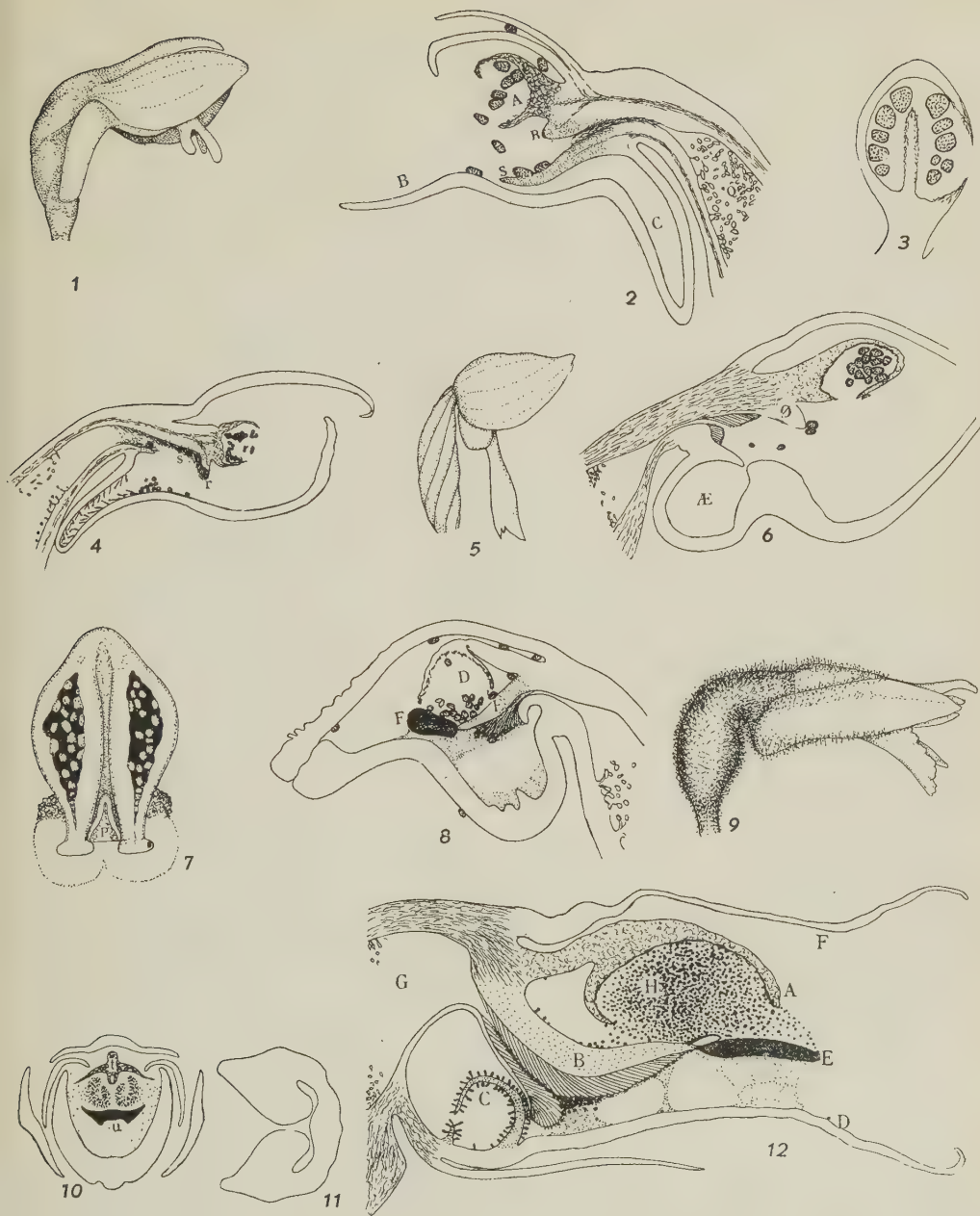
Figs. 1-12 — Fig. 1, flower of *Leucorchis albida*. ×5. Greenland. Fig. 2, *Leucorchis albida*, median longitudinal section of flower. ×23. Greenland. (*A*, anther; *B*, labellum; *C*, spur; *R*, rostellum). Fig. 3, l.s. opened anther from a bud of *Leucorchis albida* showing loosely arranged massulae. ×23. Greenland. Fig. 4, median longitudinal section of flower-bud of *Habenaria hyperborea*. The anther is open and the massulae have been liberated (*r*, rostellum; *s*, stigma with pollen). ×8. Greenland. Fig. 5, flower of *Coeloglossum viride*. ×3. The Faroe Islands. Fig. 6, median l.s. of bud of *Coeloglossum viride* showing open anther with the massulae falling out (*Æ*, spur). ×9. The Faroe Islands. Fig. 7, open anther from flower bud of *Leucorchis albida*; the rostellum *P* is very short. ×23. Greenland. Fig. 8, median longitudinal sections of the flower bud of *Goodyera repens*. Anther is already open, massulae having fallen down on stigma; note rostellum (*E*) bearing a viscid gland (*F*) at its apex. ×10. Denmark. Figs. 9-12, *Spiranthes spiralis*. Denmark. Fig. 9, flower almost closed. Fig. 10, t.s. flower; note the open anther (*t*) and pollen falling out round rostellum (*u*). ×13. Fig. 11, t.s. base of labellum; the two inwardly turned flaps cover the entrance of the spur. ×17. Fig. 12, t.s. of flower (*A*, anther; *B*, rostellum; *C*, flap at entrance of spur; *D*, labellum; *E*, viscid gland; *F*, perianth; *G*, cavity of ovary; *H*, pollen). ×17.

with glandular hairs, possibly acting as organs secreting nectar.

These two strange bodies belonging to the spur recall the flap entrance of the bladder of *Utricularia*. However, they are not wholly unknown in the orchids, for something corresponding to them is found in *Coeloglossum*, *Herminium* and a few tropical genera (cf. Pfitzer, 1889, p. 116).

If a humble-bee puts its proboscis into the flower of *Spiranthes* (Fig. 12), it will meet still other obstacles before reaching the honey. First the proboscis must penetrate the mucilage which surrounds the viscid gland *C* at the apex of the rostellum *B*. Next it has to enter the viscid substance, which is found on the stigma and which is capable of receiving the pollen that may possibly be brought from other flowers. Finally the entrance to the spur is almost barred, partly by the lower flap of the stigma and partly by the two folding doors *C* mentioned above.

Moreover, the flower of *Spiranthes* is also remarkable on account of the structure of the rostellum *B* which does not usually function as a stigma in most other orchids but is here provided with stigmatic papillae quite up to the apex, thereby offering a considerably increased chance of autogamy.

In its effort to reach the nectar a visiting insect will hardly miss touching the mucilaginous viscid gland *E* and tearing it loose; for it is situated very loosely on a very thin stalk. Thus it is fairly certain that if a flower shows a viscid gland in its original place, it has not been pollinated by an insect.

A cross-section (Fig. 10) shows that the anther dehisces along both sides, after which the pollen grains, which are not united into massulae, fall down around the edge of rostellum *u* and out into the interior of the flower, where they adhere to the mucilage of the stigma. The rostellum is particularly certain to be sprinkled with pollen, because it bears papillae quite up to the apex, which in turn is situated just outside the pore of the anther from where the pollen is being discharged almost at the same time as the opening of the flower.

The entrance to the flower is so narrow that it just gives room for the proboscis of a bee, and in the middle of this narrow opening is found the viscid gland.

It thus seems as if the flower can be both autogamous and entomogamous; this may be accomplished almost simultaneously, if insect visits are sufficiently frequent. However, if the insects fail, as in the Danish climate, where the flowering takes place rather late in the season, the plant will pollinate itself.

EPIPACTIS (Figs. 13-16) — Many good descriptions and pictures already exist of the conditions of pollination in various species of this genus (*see* Ziegenspeck, 1934). *E. palustris* and *E. persica*, for instance, have proved to be autogamous; and the same applies, according to Nannfeldt (1946), to *E. leptochila* Godf. whose pollen grains remain separate from one another.

Unlike this, the majority of the pollen grains are more coherent in our other species of *Epipactis*, and the mechanism of their pollination is not fully understood in such cases. My observation on *E. purpurata* Sm. and *E. helleborine* L., generally considered entomogamous (several investigators, such as Darwin, having observed visits by various insects), are given below.

I came to doubt the value of insect visits for various reasons; primarily because in Denmark I noticed such visits only occasionally, although wasps are plentiful. Nevertheless, there is as good fruit set as in the autogamous species mentioned above. And the size of the numerous young fruits decreases uniformly from the lower to the upper end of the inflorescence, as if pollination took place automatically once the flower reached a certain stage of development. Such is not the case in the entomogamous species of orchids.

In nearly median microtome sections passing through its middle, the flower showed some remarkable features: the rostellum is provided with papillae along the whole front up to the viscid gland, a rare phenomenon in orchids where the rostellum does not, as a rule, show any germinating pollen. Further the rostellum shows a sharp transverse fold *G* (Fig. 15) which is pressed inwards against the wall of the anther and remains like a stopper in the hole made there so

that the pollen does not fall out until the flower starts opening. The labellum shows a conspicuous curve outwards after which the fold pulls out from the hole in the wall of the anther, so that the pollen has now a free access into the centre of the flower which contains the stigma.

The masses of pollen do not form densely cohesive, large lumps. From the edges of the latter a gradually increasing number of tetrads is liberated, which fall out one by one through the previously mentioned hole lying just above the apex of the rostellum. Gradually the size of the aperture in the wall of the anther enlarges and increasing quantities of pollen are liberated, pouring out and round the edges of the rostellum. This is densely studded with long papillae (Fig. 14) which immediately bind the pollen in a big drop of mucilage spreading all over the styles situated below.

This strange form of autogamy takes place directly before or a little after the opening of the flower. Therefore, only small quantities of pollen are wasted and the majority of the slowly liberated tetrads are captured with almost auto-

FIGS. 13-18—Fig. 13, flower of *Epipactis purpurata* (*n*, anther; *o*, pollen; *p*, rostellum; *q*, labellum). ×2. Denmark. Fig. 14, *Epipactis helleborine*, t.s. of rostellum (*R*) and rest of anther (*S*). ×8. Denmark. Fig. 15, t.s. flower bud of *Epipactis purpurata*; the wall of the anther has burst and the pollen has begun falling out (*G*, fold of labellum *H*; *T*, rostellum; *Q*, wall of anther). ×9. Denmark. Fig. 16, Newly opened flower of *Epipactis helleborine*, median l.s. of anther *L* and rostellum *J* with viscid gland *K*; the anther is nearly emptied and the stigma is pollinated by the flower's own pollen. ×12. Denmark. Fig. 17, middle part of median l.s. of open flower of *Anacamptis pyramidalis* pollinated by insects (*I*, cavity of spur; *J*, rostellum; *L*, cavity of ovary; *M*, labellum; *N*, anther; *O*, stamen; *P*, perianth; *R*, stigma; *V*, viscid disc). The rostellum (*V-J*) closes the entrance of the spur, covering the stigma *R*. ×10. Denmark. Fig. 18, *Cephalanthera rubra*, median l.s. of bud showing autogamy; the anther *Y* has discharged nearly all its pollen, some of which is lying in the mucilage *X* on the stigma (*Z*, rostellum; *U*, labellum). ×4. Denmark.

matic certainty by the large viscous drop lying in the narrow space of the interior of the flower bud. When at last the flower has opened completely, the stigmas are already covered with the flower's own pollen which germinates quickly and sends down numerous pollen tubes through the stylar canal.

In autogamy the viscid gland remains at its place at the apex of the rostellum. In completely opened flowers, however, it is often found to have vanished, for both the rostellum and the anther dry up and perish. There is, however, hardly any doubt that an open flower may be pollinated by insects with pollen from other flowers. But this is probably of minor importance, as the flower is not self-sterile and insects arrive too late in the season.

In occasional flowers it is of course possible that the anther does not yield much pollen until it has already opened. In such cases entomogamy may not be quite valueless.

E. atropurpurea behaves mainly like the other species of the genus. The pollen is so loose in the anther that it readily spreads out on the inner surface of the bud during the process of preparing the flower for study. Similar spreading apparently occurs in nature and it begins prior to the opening of the flower. However, all the pollen is not liberated at once, when the anther is opened along the surface facing the rostellum, but a little at a time during the entire period of the flowering falls just along the edges of rostellum which are provided with papillae (Fig. 14) and covered by a very sticky mucilage.

Although this mechanism of bud autogamy acts with great efficacy, pollination by insects is not completely excluded. This is evidenced by the fact that this species hybridizes in nature with *Cephalanthera rubra* (Schulze, 1894).

CEPHALANTHERA RUBRA — Darwin's (1904) classic study has shown that *Cephalanthera damasonium* and *C. longifolia* are also autogamous like *Epipactis*.

C. rubra has, however, not been sufficiently investigated. From the colour, size and conspicuousness of the flowers it would seem natural to suggest insect pollination, and Ziegenspeck (1934) has actually ascribed to *C. rubra* and *Epipactis palustris* " a sehr ausgepragter Insektenbestäubung ". However, he did not actually notice insect visits and, as will be shown below, his supposition is incorrect. Even in the closed bud shown in a nearly median longitudinal section (Fig. 18) the wall of the anther has opened and is almost completely lost on the side towards rostellum which is so short that the anther protrudes high above it. The rostellum bears at apex a viscid gland which is papillate throughout its length and is covered by mucilage.

Even in bud nearly all the pollen pours down from the anther; the nearest place for it to fall is beyond the edges of the rostellum, where it is captured by the mucilage of the stigmas. Also some pollen gets stranded on the labellum and may be liberated later when the wind shakes the plant.

Pollen may be found all over the inner surface of the flower, and part of it is certainly washed away during the process of preparation of the flower for study. This proves its loose and easily transportable nature.

When the flower opens, the lip is frequently turned upwards and then the pollen, lying in the inequalities of labellum, may often be liberated, and fall on the stigma.

A good deal of pollen is often found on the viscid gland, which may possibly function if the completely opened flower is visited by insects. This is only accidental but may be of importance as shown by the formation of the hybrids with *Epipactis purpurea*, already mentioned above.

The normal mode of pollination in our three species of *Cephalanthera* and six of *Epipactis* is, however, by bud autogamy.

ANACAMPTIS PYRAMIDALIS — This species has been previously examined by Darwin (1904) and others and stands in contrast with the autogamous orchids mentioned above in its inability to pollinate itself and its dependence on insects.

Fig. 17 shows a nearly median longitudinal section of a fully open flower showing several interesting details. The

rostellum is remarkable because of its curvature so that it is transversely placed in the flower, thereby partially closing the entrance of the spur which can only be forced open by a powerful insect. Such a strong mechanical encroachment is, however, necessary in order to expose the stigma to the reception of the pollen. Immediate autogamy is, therefore, improbable. Indeed in the open and unpollinated flower the pollinia are intact and coherent and all the massulae were found in their original places. Moreover, the rostellum is remarkable in the viscid disk *V* being formed by the apex as in the acrontonous orchids.

Epipogium aphyllum, which I examined, also seems incapable of autogamy.

Discussion

The ingenious adaptations for insect pollination shown by tropical orchids are lacking in several species which are autogamous (Kirchner, 1922).

With a decrease in the number of insects towards the north the percentage of autogamous orchids is found to increase greatly. Thus even Darwin was aware of no less than 10 autogamous European species. Those occurring northernmost are: *Plantanthera hyperborea*, *Epipactis persica*, *Cephalanthera damasonium*, *Listera ovata*, and *Neottia nidus avis*.

Later writers have considerably extended this list. Thus Kirchner (1922) added *Epipactis palustris*, *Coralliorrhiza innata*, *Herminium monorchis*, *Listera cordata*, and *Liparis loeselii*. In all he found fifteen autogamous orchids in Europe. In addition, he has given a long list of tropical autogamous species, classifying them according to the manner in which pollination takes place.

Nine other species have been shown to be autogamous in the present investigation. Of these, *Habenaria* extends farthest to the north in the Atlantic area.

In Denmark there are eighteen genera of orchids. The majority of these show autogamy. *Orchis* is the chief entomogamous genus; but even in this Martens (1926) occasionally found autogamy. The following species require further study: *Cypripedium calceolus* and *Hammarbya*

paludosa. I have not been able to investigate sufficient material of these rare species. But judging from what is already available it seems as if about half (19) of the orchids of Denmark are autogamous; and perhaps the number may be still larger.

Within the nine species examined here, the mechanism of autogamy functions in a remarkably similar manner and begins to act at almost the same stage of the development of the flower, i.e. just when it is beginning to open, but some time before any insects can enter the flower. However, autogamy also continues even after the flower has opened.

The wall of the anther dehisces by a slit along the inside. Mostly the pollen is loose and dry. It gradually falls out from the anther and, as the flower bud is nearly horizontal at the pollinating stage, pollen becomes scattered along the edges of the rostellum and inwards to the central cavity of the bud where it is captured by the mucilage secreted by the stigma.

In some species the rostellum acts as a stigma; it is densely papillate (*Epipactis, Cephalanthera, Spiranthes, Habenaria*). In other species the two other stigmas are situated at the apex of long styles (*Herminium, Gymnadenia albida*, and *G. hyperborea*), which protrude to the place where pollen falls out from the anther.

The autogamous period of the flower may be terminated either by an insect removing the remainder of the pollinia or after all the pollen has been discharged from the anther. The latter happens when the walls of the anther have withered, which synchronizes with the time when the stigma as well as the rest of the flower are dead.

Depending on the circumstances, the flowers may, therefore, be pollinated either by insects or they may be autogamous. The latter is the only method in the case of plants inhabiting places where suitable insects are not present in sufficient quantities. It is also possible that certain flowers become pollinated in both ways simultaneously. For during the vigorous shakings caused by an insect visit, a much greater quantity of the flower's pollen will be liberated and fall on the mucilaginous drop of the stigma.

It is, however, important that autogamy generally begins before the visit of an insect. The plant's own pollen germinates immediately after it has reached the stigma; so the plants can scarcely be considered to be self-sterile. Entomogamy is, therefore, usually only an accident without much value, because it occurs too late.

Nearly all the species here investigated have small, partly closed flowers. They, however, possess both fragrance, colour, and honey and several of them are often visited by insects. In more southern climates entomogamy is perhaps of greater importance than in the north. Even here in Denmark it may happen that in certain flowers autogamy occurs somewhat later than is normal, so that the two different modes of pollination occur almost concurrently. Generally, however, nearly all the species mentioned here are autogamous and the effectiveness of this manner of pollination is demonstrated by almost all the flowers developing fruit even in the northernmost and least favourable localities.

Summary

1. The conditions of pollination have been investigated in the following orchids: *Leucorchis* (*Gymnadenia, Habenaria*) *albida, L. hyperborea, Coeloglossum viride, Goodyera repens, Spiranthes spiralis, Epipactis helleborine* (= *E. latifolia*), *E. purpurata, E. atropurpurea*, and *Cephalanthera rubra*.

2. Some of the pollen lies loose in the open anther and falls down on the stigma immediately before the flower opens. The pollen germinates quickly and there is little or no self-sterility.

3. Since part of the pollen remains in the anther this can be transported by insects, but entomogamy is not of much importance as it occurs too late to be useful.

4. The species produce abundant fruit in regions and at places where insects are absent.

5. Bud autogamy is the normal form of pollination. It functions with great efficacy and is often continued during flowering.

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THE EMBRYO SAC OF *BUTOMUS UMBELLATUS* L.

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Introduction

Butomus is a monotypic genus, with the single species *B. umbellatus*, belonging to the small family Butomaceae, having four genera and nine species (Index Kewensis 1893 a to 1938). Embryo sac development for one member from each genus has been studied since 1902 and Holmgren (1913) reported *Butomus* as having the "Normal" or "Polygonum" type.[1] A restudy seemed desirable because of differences reported for the four genera.

Hall (1902) studied *Limnocharis emarginata* and stated that "there is a tapetum cut off by the archesporial cell, such as is described by Campbell (1898) for *Naias* and *Zannichellia*. In *Limnocharis*, however, the tapetal cell is without a wall, and it is pushed towards the apex of the sac where it disappears in the later stages of development. The large cell left after the formation of the tapetum becomes the embryo sac without further division." In other words, *Limnocharis* has a tetrasporic origin. In 1914 Nitzschke reported a tetrad of megaspores; this would mean a monosporic origin. Johri (1938 b) has shown that the development is bisporic and that Hall's (1902) " tapetal " cell is really the upper dyad cell.

Hydrocleis nymphoides (= *H. commersonii*) was reported by Suessenguth (1920) to have a " Polygonum type " of embryo sac formed from the chalazal nucleus of a tetrad of megaspores, even though he did not actually see this. On the other hand, Johri (1938 a) has shown that this embryo sac is also bisporic.

This bisporic origin again appears in a third genus of the family, *Butomopsis lanceolata* (Johri, 1936). The single hypodermal archesporial cell functions directly as the megaspore mother cell without cutting off any wall cell. The upper cell of the dyad resulting from meiosis I degenerates whereas the lower gives rise to the nuclei of the embryo sac, thus making its derivation bisporic. Johri reports that the lower nucleus enters meiosis II and the primary micropylar and chalazal nuclei so formed are separated by a membranous partition near the chalazal end. The chalazal nucleus undergoes no further division and consequently there is no lower polar nucleus. The egg apparatus and upper polar nucleus are formed by two mitotic divisions of the primary micropylar nucleus. This type of embryo sac is, therefore, bisporic with reduction of the number of nuclei at the chalazal end.

The fourth member of .the family, *Butomus umbellatus*, is the only one that now remains with a monosporic origin (Holmgren, 1913). The development of the embryo sac has been carefully checked and it is hoped that this fairly complete developmental story will round out the previous reports of Buchenau (1857), Vesque (1878), Ward (1880) and Holmgren (1913), and leave no doubt that the embryo sac is monosporic in origin and of the " Polygonum " or " Normal " type in development.

Material and Methods

All the material was collected between the months of June and August from the

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1. The terminology used in this paper follows that adopted by Maheshwari (1937 to 1950) unless otherwise stated.

mud flats bordering the St Lawrence river at Ile Perrot, Quebec.

The top of the ovary was excised and the lower portion immediately placed in a bottle containing equal parts of absolute alcohol and glacial acetic acid. To ensure rapid penetration of the, fixative most of the air was withdrawn from the bottle with the help of a hand pump. On return to the laboratory the fixative was replaced by a fresh solution and the bottles pumped again.

The celloidin method was used for embedding and the material was sectioned at 16 μ for young stages and 18-24 μ for later stages. Numerous sections were discarded in the preliminary study and there remained just over 800 sections for more detailed examination. Nearly all of these were longitudinal.

About 20 of these preparations showing early post-fertilization stages were stained with ruthenium red to ascertain whether certain specific walls were present. The success of this technique was doubtful although, when coupled with varying amounts of Heidenhain's iron haematoxylin, nuclear staining was most satisfactory (Fig. 41).

In addition to the well-known smearing techniques, a semi-permanent Venetian turpentine method was used for post-fertilization stages. After using aceto-carmine to stain the partially broken ovules, it was flushed off the slide with two changes of Venetian turpentine. The coverslip was then added using a drop of the Venetian turpentine as a mounting medium and further squashing was done at discretion. Fig. 27 is drawn from one of these smear preparations.

For most of the study the sections were mordanted for 30 minutes with iron alum and stained for 12 hours with Heidenhain's haematoxylin, after which they were destained. This procedure was followed throughout, even for the very young stages; hence if all the cells within the developing nucellus destained to the same degree and were all of the same size, it was assumed that there was no differentiation at that stage. The sections were of a blue colour and not grey as is usually recorded in the literature.

Observations

Butomus has six carpels which are free for most of their length, but connate near their base. Therefore, longitudinal sections may pass through one, two or three carpels.

Protuberances, which are destined to become ovules, arise from the inner carpellary walls. Before any differentiation can be seen within these protuberances, microsporogenesis is complete and a tapetal periplasmodium (Maheshwari, 1950) surrounds the isobilateral tetrad of microspores. Meanwhile, the protuberances have enlarged from 35 to about 75 μ. All the cells stain with equal intensity and they are actively dividing. The archesporial cell is visible only after the rounding up of the microspores.

Figs. 1 and 2 show archesporial cells. The young ovule at this stage measures approximately 100 μ and for the first time the direction of growth changes. Whereas in the earlier stages growth was towards the centre of the carpel, it has now changed so that the ovule is being carried upward. The first signs of the inner integument can be detected in Fig. 1 and more clearly in Fig. 2; it arises approximately in line with the lowest extremity of the archesporium.

This archesporial cell does not cut off a " tapetal " cell as was stated by Holmgren (1913) but enlarges to give rise to the megaspore mother cell directly (Fig. 3). Thus Fig. 3 follows Fig. 1, and not Fig. 2 as Holmgren's report would suggest.

Ovules with archesporial cells covered by only one row of nucellar cells are fairly rare, and such ovules have a slightly different type of development. The usual series is shown in Figs. 1 and 3-7. In other words, the archesporial cell ordinarily differentiates in a hypodermal cell and is covered by two rows of nucellar cells.

In the microsporangium the microspores become surrounded by the " tapetal " periplasmodium. An exine is distinct and within these cells certain changes are evident. The cytoplasm becomes extremely vacuolate and the nucleus moves to one wall of the microspore and there undergoes a mitotic
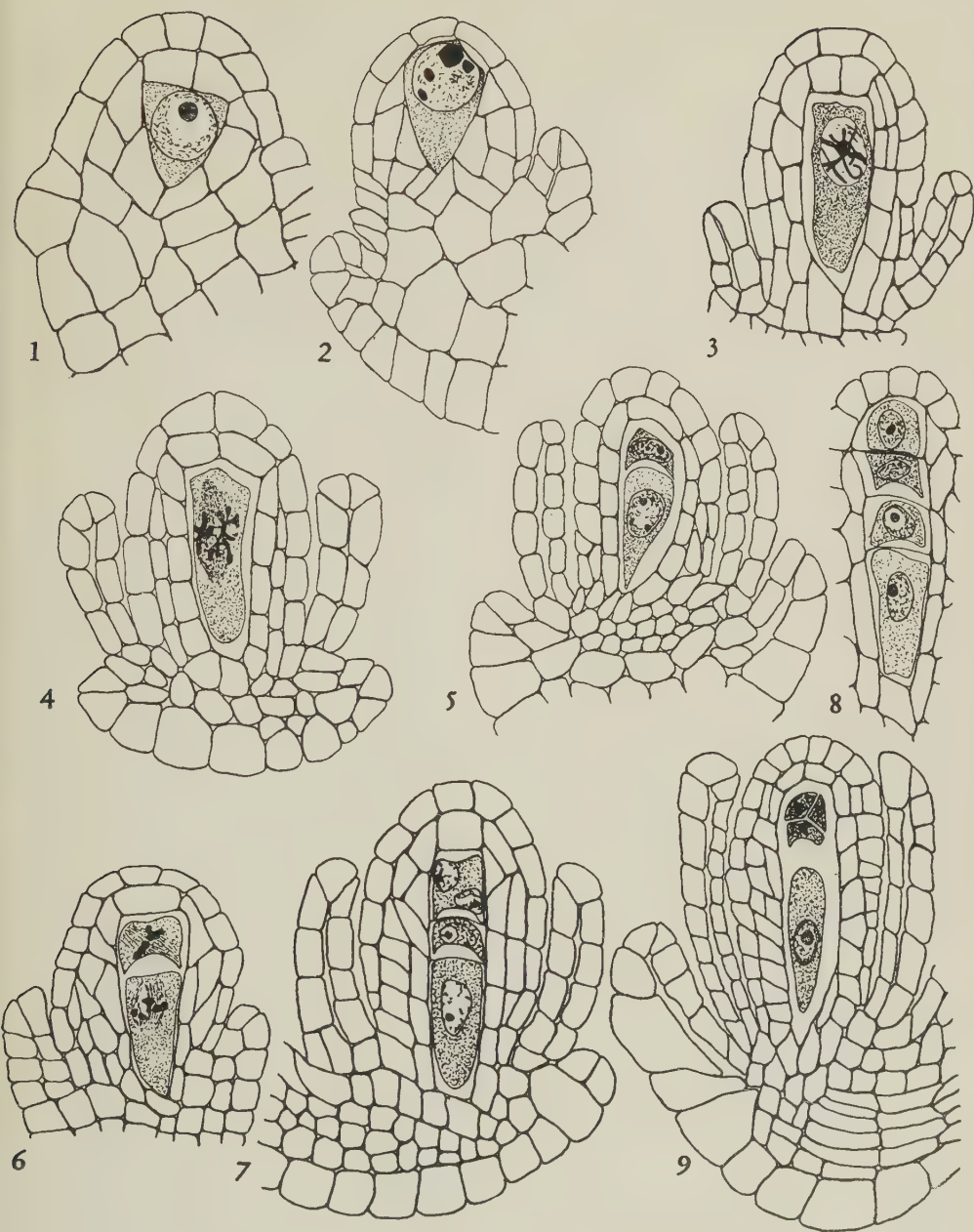
FIGS. 1-9 — Megasporogenesis. (In all the figures the micropylar end is uppermost.) Figs. 1, 2, archesporial cells differing in origin. Figs. 3, 4, megaspore mother cell in prophase I and metaphase I. Fig. 5, dyad resulting from meiosis I. Fig. 6, metaphase II. Figs. 7, 8, T-shaped and linear arrangement of megaspores. Fig. 9, functional megaspore with the three degenerating micropylar megaspores. ×700.

division. All stages from prophase to the end of this division occur in the same microsporangium. The ovules of the same flower show the archesporium now enlarged considerably with the megaspore mother cell in early prophase I (possibly zygotene — Fig. 34). Several growth changes have occurred so that the ovule assumes an anatropous condition. Measurement of the ovule and its funiculus gives an average value of 170 μ. In other words, it has increased an average of 70 μ since differentiation was first observed. This increase has not been confined to the funiculus, for the cells lateral to the archesporial cell have also multiplied and lengthened. No cells have been added above the archesporial cell. Figs. 3 and 34 show the position of the integuments at this stage. The cells of the inner integument have multiplied so that the tip of the integument is approximately in line with the nucleus of the megaspore mother cell. The position of this integument is extremely valuable in sectioning, for where one is not able to see the unstained nucleus, the position of the integument acts as a guide. The origin of the outer integument can also be observed. If the section has the funiculus included, as in Figs. 7, 9 and 11, only one side of the outer integument is seen. If, however, the longitudinal section does not pass through the funiculus, both sides of the outer integument may be observed (Figs. 4, 34).

The megaspore mother cell has been seen in numerous stages of the first division. Two stages of prophase I are represented in Figs. 3 and 34. In the former, the chromonemata are contracting and the matrix is accumulating as diplotene is approached.

Another stage of meiosis I is shown in Fig. 4. The meiotic figure is situated nearer the micropylar portion of the megaspore mother cell, so that when the cell plate, and later the cell wall, is formed the lower cell of the dyad is larger and has more cytoplasm than the upper cell (Fig. 5).

Each dyad cell usually undergoes meiosis II, but some sections suggest that occasionally the upper cell degenerates without dividing. The spindles are usually developed in planes at right angles to one another resulting in a " T-shape " arrangement of megaspores (Figs. 6, 7 & 9). Occasionally the linear arrangement is observed (Fig. 8). The obvious explanation for these two types of arrangements is one of space. Where two rows of nucellar cells lie above the upper figure, a " T-shape " results. Where only one row of nucellar cells is present, there is more available room and the linear arrangement is possible.

The three micropylar megaspores of the tetrad degenerate fairly quickly and the chalazal megaspore becomes functional (Fig. 9). Before this functional state is reached, there are numerous growth changes. The early degeneration of the micropylar megaspores is suggested in Fig. 9. No walls could be detected and degeneration apparently involves the dissolution of the " weak " walls which were difficult to see in many sections (Fig. 7). Although degeneration is far advanced in Fig. 9, there must be quite a time lapse before the chalazal megaspore becomes functional, because, by the time the first mitotic division is in progress, the inner integument almost completely invests the inner structures and the nucellar cells have lengthened in the long axis of the ovule and allowed for an increase in the area of the uninucleate embryo sac cell.

The term " uninucleate embryo sac cell " seems necessary since the functional megaspore occupies not only its own cell, but, due to the dissolution of the cell walls separating the non-functional megaspores, it spreads into the available space beyond. The uninucleate embryo sac, therefore, includes the area previously occupied by both the degenerating megaspore nuclei and the functional megaspore. Also this cell, although it enlarges at the expense of other cells, can be traced through to the most advanced stage (Figs. 9-18). In addition, later stages can be referred to in terms of the two-nucleate embryo sac, the four-nucleate and eight-nucleate embryo sacs and finally the mature embryo sac when the organization of the nuclei is complete. This is far more convenient than repeatedly using such cumbersome terminology

as "two-nucleate stage of the developing embryo sac".

As the nucleus begins its first mitotic division within the uninucleate embryo sac, the cytoplasm is characteristically disposed in that a vacuole is found on either side of the nucleus in the direction of the long axis of the cell. Division results in the formation of two nuclei (Fig. 10) which migrate to opposite poles. A vacuole is formed between these two nuclei as they move apart, and eventually the cytoplasm at the poles is connected by a very thin peripheral bridge of cytoplasm (Fig. 11).

The primary chalazal and the primary micropylar nuclei undergo two further simultaneous mitotic divisions to produce the eight-nucleate embryo sac (Figs. 11-15, 35). It may be added that in Fig. 35, although all the mitotic figures are not in focus, their presence has been substantiated. Fig. 14 was reproduced from the same section.

In the formation of the eight-nucleate from the uninucleate embryo sac, one change has taken place at the micropylar end. Cytoplasm is slowly lost from the layer of nucellar cells immediately above the embryo sac and this row of cells gradually becomes flattened against the one remaining layer of the nucellus (Figs. 13-17). Presumably this would not occur if the embryo sac were to arise from the linear arrangement of megaspores (Fig. 8).

Measurements show an average increase in the length of the embryo sac of 9-10 μ (i.e. from 73 to 80 μ) from the prophase of the uninucleate to the eight-nucleate condition. This difference in size is accounted for by the loss of one row of nucellar cells.

The eight nuclei become organized in the usual manner. Three nuclei of the micropylar quartet give rise to the " egg apparatus " whereas the fourth forms the upper polar nucleus. At the chalazal end, it is difficult to state dogmatically that three antipodal cells are formed. Three antipodal nuclei with their own investment of cytoplasm are definitely present, but the point at issue is whether they are separated by walls. After a careful study of numerous ovules it seems

that in nearly all cases a " weak " wall is formed as represented in Figs. 15, 16, 17, 19, 21 and 22. The photomicrographs shown in Figs. 36 and 41 support this conclusion. The fourth nucleus of this quartet is the lower polar nucleus.

The egg apparatus is composed of two synergid cells and an egg cell. These cells are seen in one view in Fig. 15 and turned through a right angle in Figs. 16 and 17. In the former case the synergid cells are practically superimposed and the egg cell lies lateral to them. In the latter the synergids lie adjacent to one another and above the egg cell. There is a tendency for the synergids to be pointed at the micropylar end, but no sign of a definite beak or " filiform apparatus " could be seen.

The synergid nuclei are the same size and lie in the cytoplasm at the upper end of the cells. Within each synergid is a vacuole at the lower end (Figs. 16, 17). The nucleus of the egg cell is larger than either of the synergid nuclei and occupies the lower part of its cell. It has a large vacuole toward the upper part of the cell (Figs. 18-20).

The two polar nuclei migrate to the centre of the embryo sac. Very often one nucleus does not migrate as far as the other, and they meet nearer the egg or nearer the upper antipodal cell. They always migrate with an aggregation of cytoplasm and when they meet they are connected to the opposite ends of the embryo sac by thin strands of cytoplasm. Large vacuoles are always evident (Fig. 16). These nuclei usually fuse to produce a secondary nucleus (Figs. 17, 36) prior to fertilization. However, in one case (Figs. 18, 39) the polars were observed as still free with a male gamete in contact with them; triple fusion would occur in this case.

The pollen tube has been seen in numerous sections and is always characterized by the taking of a very deep stain. During its entry into the embryo sac it crushes one of the synergids. The remaining synergid persists until the first division of the primary endosperm nucleus. It is difficult to state whether its cell walls are always retained as shown in Fig. 20. In Fig. 18 observation was
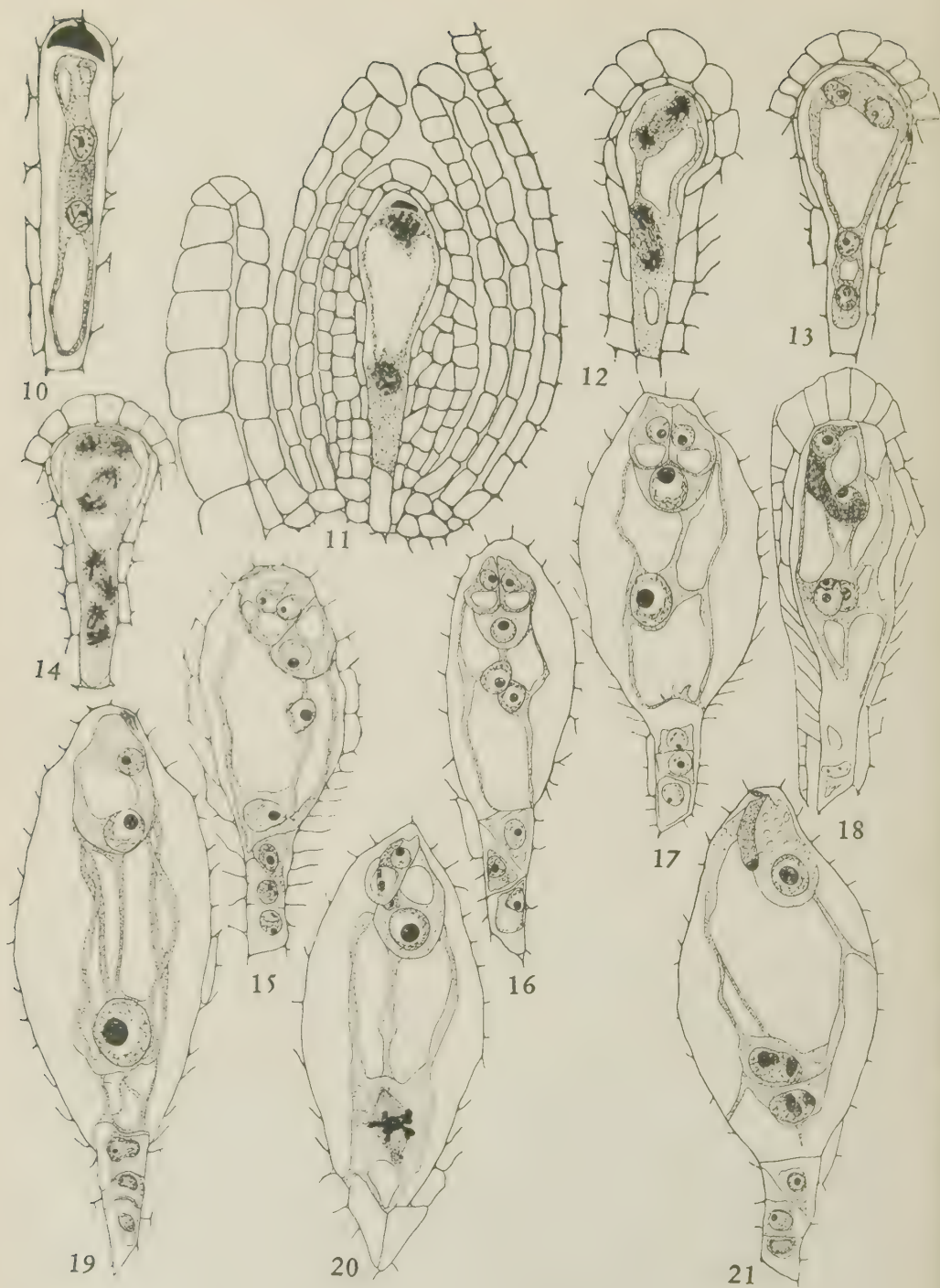
FIGS. 10-21

difficult due to the position of the pollen tube or rather to the plane in which these sections were made. Had they been sectioned longitudinally as here but in a plane perpendicular to the paper, the pollen tube would then lie to the right (or left) of the remaining synergid and it would pass completely through and destroy the other synergid. This has actually been seen in another section.

Two male gametes have been observed. One section revealed the presence of one male gamete in contact with the second-ary nucleus (Fig. 37), resulting from the previous fusion of the polar nuclei. Although these nuclei were in contact with one another, the membranes of each were still distinct. It would appear from measurements that the ' secondary ' nu-cleus had a volume of the order of 40 to 50 times as great as the male gamete. This male gamete consisted of a nucleus with a very small nucleolus. As it was embedded in the cytoplasm surrounding the " secondary " nucleus, it could not be determined whether it possessed any cyto-plasm of its own.

The other male gamete within this embryo sac was resting above the nucleus of the egg cell. It is shown as the dark spot almost out of focus at the upper end of Fig. 37. It seemed to be all nucleolus due to the intensity of the stain and around it was a narrow colourless area (as in Fig. 38). This nucleus was em-bedded in a dark staining cytoplasm which was similar to, and in contact with, that of the pollen tube mentioned previously. As a result, it is impossible to say if the cytoplasm seen was intrinsically part of the male gamete or of the pollen tube. The pollen tube of Fig. 37 is also out of focus but may be seen as a shadow ex-

tending out of the figure at the upper extremity.

A somewhat later stage is represented in Fig. 18 (reconstructed from Figs. 38 & 39). For *Butomus*, the fusion of the polar nuclei usually occurs before the entry of the pollen tube.

Early post-fertilization stages indicate that the nuclear contents of the male gametes merge with the contents of the nuclei that they entered. The fertilized egg becomes the zygote, whereas the centrally suspended nucleus is the " pri-mary endosperm nucleus ".

The primary endosperm nucleus (Fig. 19), with an aggregation of cytoplasm, moves to the chalazal end of the embryo sac, being connected to the micropylar end by " threads " of cytoplasm. The nucleus undergoes a division. Fig 20 shows such a division in metaphase and Fig. 40 in anaphase, while Fig. 21 illus-trates the completion of the division.

A membrane is formed between the upper and lower endosperm nuclei and always appears curved (Figs. 21, 22, 41 *et al.*). No wall as such can be seen passing across to the lateral walls of the embryo sac as reported by Holmgren (1913). However, the lower endosperm nucleus does not divide again, but en-larges and behaves quite differently from the upper endosperm nucleus. This beha-viour places it in the group with the " Helobial type " of endosperm forma-tion.

The cytoplasm separating the upper endosperm nucleus from the dividing membrane becomes vacuolate (Fig. 22). This nucleus migrates back to a central location (Fig. 41) so that it occupies approximately the same position and appears similar to the primary endosperm

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Figs. 10-21 — Female gametophyte, fertilization and endosperm. Fig. 10, early two-nucleate embryo sac. ×700. Fig. 11, primary micropylar and chalazal nuclei in late prophase and sepa-rated by a central vacuole. ×600. Fig. 12, telophase of the second mitotic division. ×600. Fig. 13, four-nucleate embryo sac. ×600. Fig. 14, telophase of third mitotic division. ×600. Fig. 15, eight-nucleate embryo sac. ×600. Fig. 16, embryo sac showing the upper and lower polar nuclei lying adjacent to each other. ×600. Fig. 17, mature embryo sac prior to fertilization. ×600. Fig. 18, syngamy; there is an unusual lag in the fusion of polar nuclei, thus allowing for triple fusion with the male gamete. ×600. Fig. 19, zygote; endosperm nucleus migrating to chalazal end. ×600. Fig. 20, primary endosperm nucleus in metaphase of first division. ×600. Fig. 21, upper and lower endosperm nuclei. ×600.
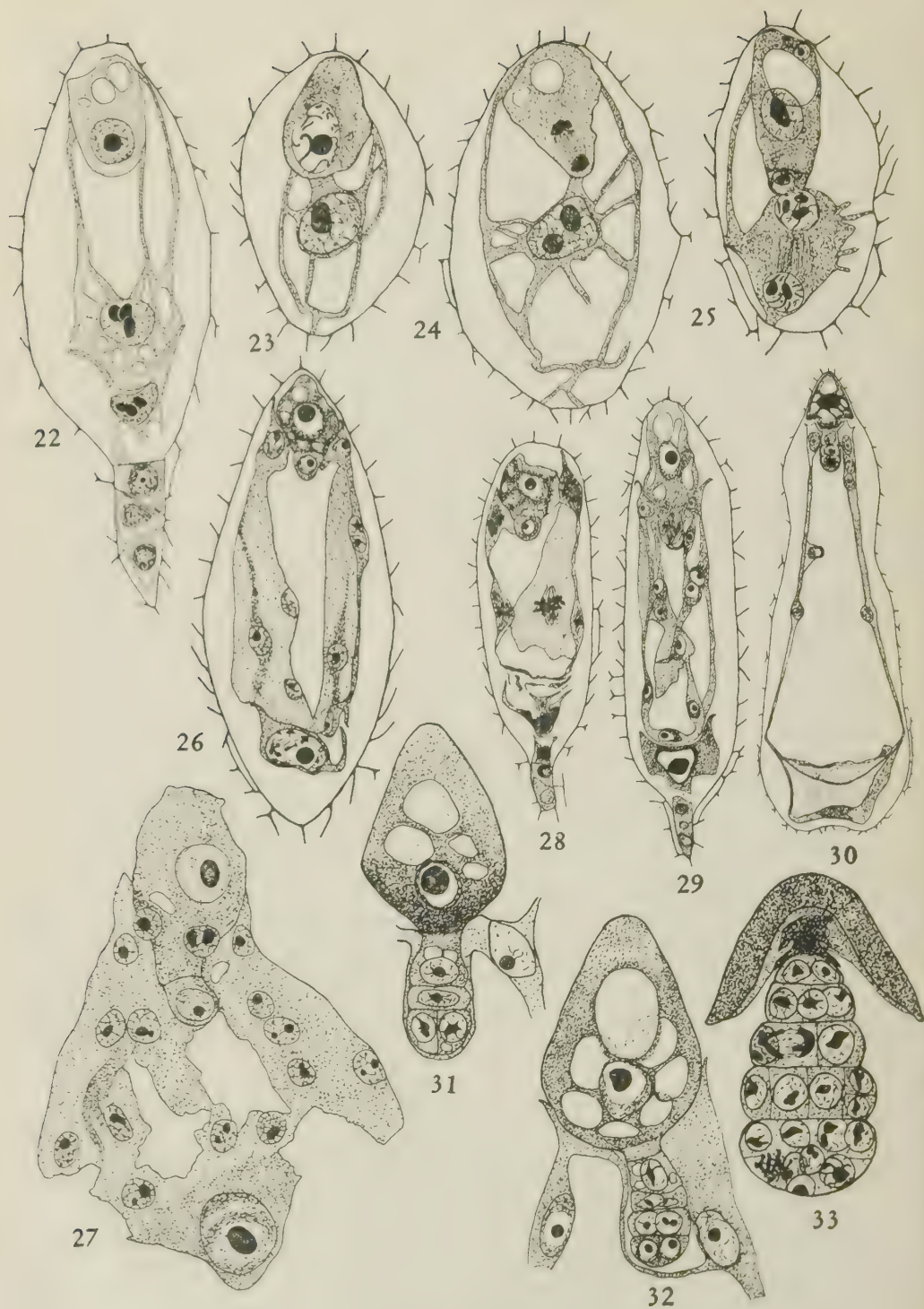
FIGS. 22-33

nucleus immediately after fertilization had occurred.

During these stages, the zygote remains inactive and one synergid cell (or nucleus) usually persists. The zygote now undergoes its first division. Figs. 23, 24 and 25 illustrate prophase, telophase and the end of the first division respectively. The two-celled proembryo consists of a large upper " basal " cell and a lower small " terminal " cell. If a synergid nucleus is still present, it is in a state of degeneracy (Fig. 25). No sign of a pollen tube remains.

The proembryo does not undergo any further divisions for some time. However, the upper endosperm nucleus divides repeatedly (Fig. 28). The three antipodal nuclei do not enlarge or show any signs of activity. The lower endosperm nucleus, however, appears extremely active physiologically, judging by its large size and susceptibility to stain (Figs. 26, 27). In contrast to the upper endosperm nucleus, which has divided repeatedly and synchronously (Fig. 28), it does not undergo further mitoses.

The smear illustrated in Fig. 27 suggests that although there may be a membrane separating the large lower endosperm nucleus from the other endosperm nuclei, this membrane is completely lost in the technique. Had there been a wall present, then in dissecting out the embryo sac and smearing, it is most unlikely that it would be lost. The cytoplasm surrounding this nucleus takes the stain very readily.

The two-celled proembryo remains as such throughout all these endosperm changes and, as soon as the terminal cell of the proembryo is seen in its first division, all endosperm divisions appear to have ceased (Fig. 29).

The endosperm nuclei are not separated by cell walls but lie " free " and are connected by strands of cytoplasm. There is one central vacuole (Fig. 26). In later stages, when the embryo sac has increased in size, the cytoplasm and nuclei lie peripherally (Fig. 30).

At the time of the first division of the terminal cell of the proembryo (Fig. 29), the lower endosperm nucleus starts to degenerate. Characteristically, the periphery and the centre of the nucleus are deeply stained and the area between completely unstained. The nuclear membrane must then disintegrate at about the time that the terminal cell of the proembryo has completed its division to give two cells in addition to the basal cell. In later stages, the chalazal structures are either absent or in a very degenerate condition.

As for the proembryo itself, the terminal cell divides to give an upper and a lower cell. The upper one divides transversely so that a linear arrangement of three cells is seen. The lowest of these three cells (Fig. 30) then divides either longitudinally to give a pair of juxtaposed cells (Fig. 31) or divides transversely to give a linear arrangement. While later stages are not pertinent to this study, they show some of the changes which occur in the formation of the embryo and the fate of the basal cell. The latter is characterized by its large size and vesicular nature, and also by a large nucleus (Figs. 30-33). Eventually the suspensor of the embryo works back into the basal cell (Fig. 33). It is expected that a single cotyledon, stem tip, root tip, and root cap will develop since embryo formation in *Butomus* basically agrees with the Sagittaria variation of the Caryophyllad type (Johansen, 1950). From Fig. 30 the great increase

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Figs. 22-33 — Endosperm and embryo. Fig. 22, upper endosperm nucleus migrating towards the centre. ×600. Fig. 23, upper portion of embryo sac with zygote nucleus in prophase. ×600. Fig. 24, zygote in telophase. ×600. Fig. 25, two-celled proembryo: upper endosperm nucleus divided. ×600. Fig. 26, large lower endosperm nucleus very different from other endosperm nuclei. ×400. Fig. 27, two-celled proembryo, endosperm nucleus and large lower endosperm nucleus, drawn from a smear preparation. ×300. Fig. 28, synchronous divisions of upper endosperm nucleus contrasted with the behaviour of the lower. ×300. Fig. 29, terminal cell of two-celled proembryo in metaphase; degeneration of lower endosperm nucleus. ×300. Fig. 30, weak endosperm and dividing proembryo. ×200. Figs. 31-33, developing embryo; note change in basal cell. ×500.
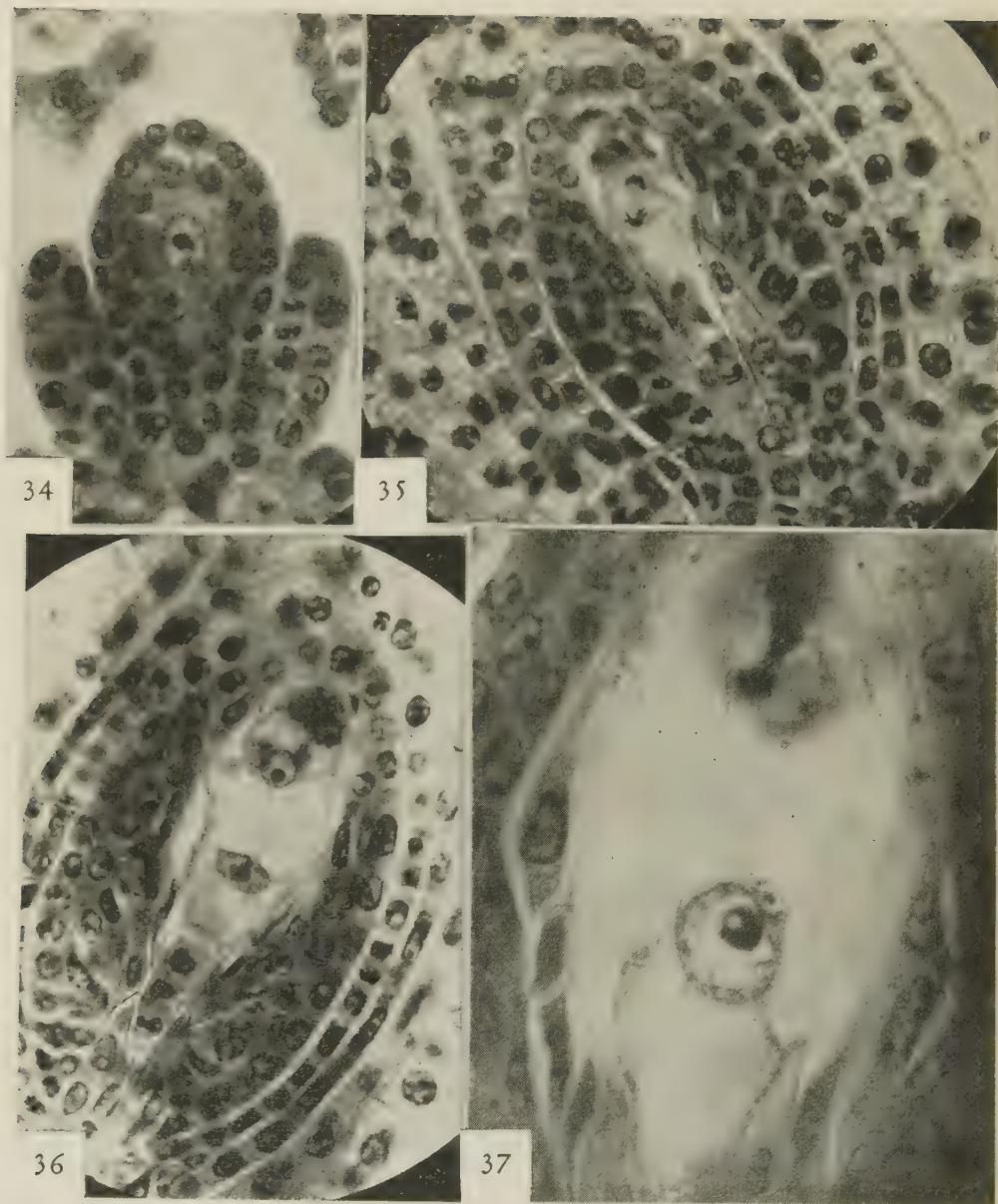
FIGS. 34-37 — Fig. 34, megaspore mother cell in prophase I. ×600.   Fig. 35, telophase of the third mitotic division. ×530.   Fig. 36, mature embryo sac prior to fertilization. ×600. Fig. 37, fertilization — one male gamete adjacent to fused polars. ×900.

in size of the embryo sac over previous stages is apparent. The walls of the nucellus become completely depleted and the endosperm appears very weak. At this stage the basal cell probably functions in an absorbing capacity.

Discussion

Johri (1938 b) implied that in all the Alismaceae and Butomaceae, the archesporium gives rise directly to the megaspore mother cell (p. 283). *Butomus*
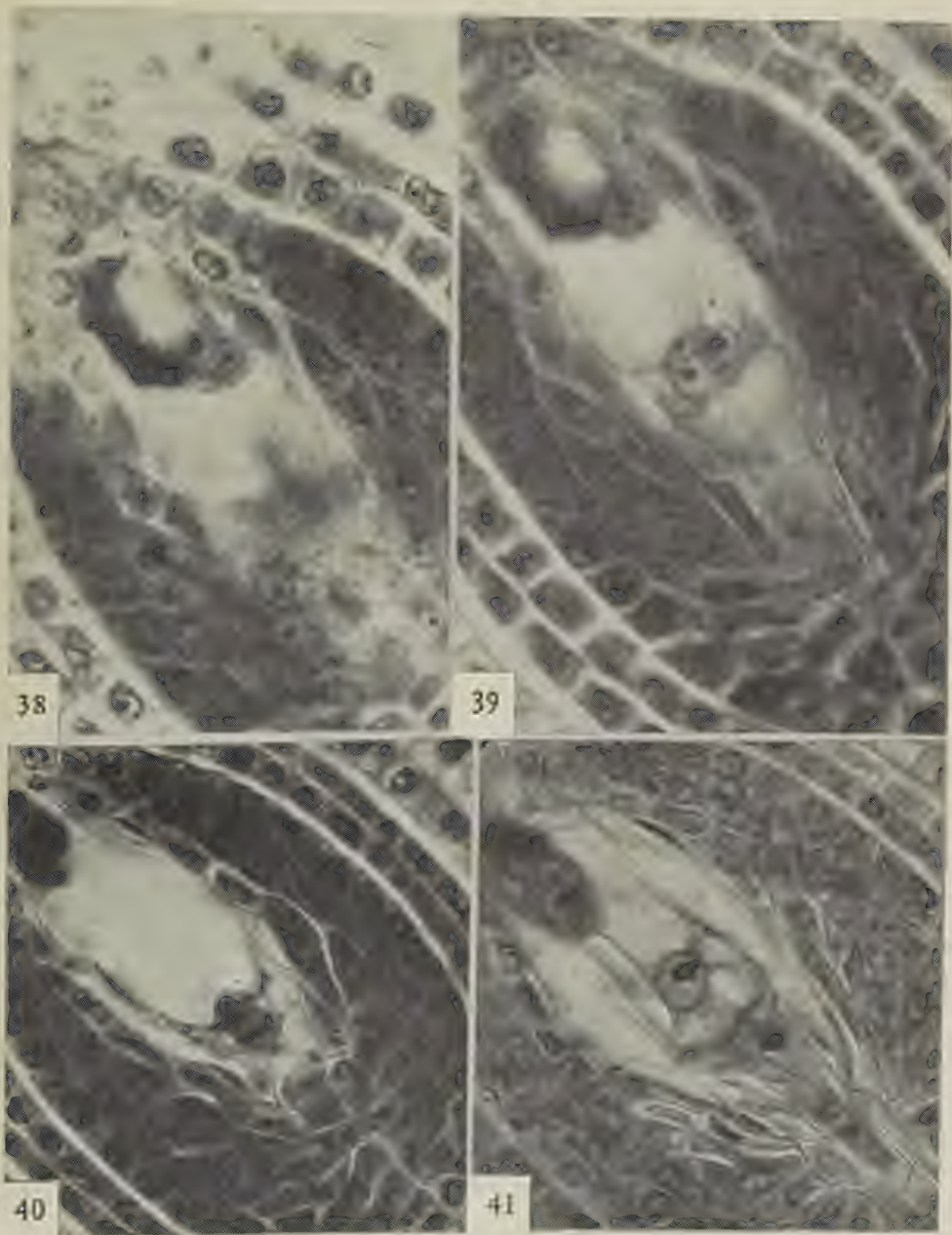
FIGS. 38-41 — Fig. 38, fertilization of the egg nucleus. ×880.   Fig. 39, triple fusion in same embryo sac as in Fig. 38. ×900.   Fig. 40, anaphase of the first division of the endosperm nucleus. ×600.   Fig. 41, zygote, upper and lower endosperm nuclei, and antipodal nuclei; shadow adjacent to the zygote is the pollen tube. ×600.

(Holmgren, 1913) was the only exception to this in forming a tapetal cell, although previously Hall (1902) thought that *Limnocharis* also formed a tapetal cell. The present study shows Holmgren (1913) to be incorrect; so *Butomus* need no longer be considered an exception.

The actual position of origin of this archesporial cell was the point at which Holmgren misinterpreted his observations. As pointed out, while it usually arises in the nucellus so as to be covered at the micropylar end by two rows of nucellar cells (Fig. 1), there are instances when it is covered by only one row (Fig. 2). The former case appears to lead to the ' T-shape " arrangement of megaspores (Fig. 7), the latter to the linear (Fig. 8). The arrangement is one of mechanics.

All genera of the Butomaceae complete the first meiotic division. Meiosis II in *Butomus* results in each cell of the dyad dividing to give four megaspores, each separated by a wall. Only the lower cell (Holmgren, 1913) is active in the formation of the embryo sac. In the other genera the upper cell of the dyad divides no further, whereas the lower cell gives rise, after the second meiotic division, to two nuclei. In reality these nuclei are megaspores, although Johri (1936) has called them the primary micropylar and the primary chalazal nuclei. These are separated . by a membranous partition near the chalazal end. This chalazal nucleus undergoes no further division in *Butomopsis* (Johri, 1936), whereas the primary micropylar nucleus gives rise to four nuclei. This has been mentioned previously, but the point which comes to mind is that this type of development does not seem very different from that described for *Butomus*. The type of development in *Butomopsis* could be regarded as similar to the " Oenothera type " in which the megaspore adjacent to the chalazal megaspore gives rise to a four-nucleate embryo sac. What has been termed the primary chalazal nucleus could with equal reason be called the chalazal megaspore. Thus one would not speak of a bisporic origin, but of a monosporic one.

The object in considering this point is not to throw doubt on Johri's (1936,

1938a, 1938b) classification, but to suggest how the type of development seen in *Butomus* could have given rise to that reported for *Butomopsis*. The occasional division of the primary chalazal nucleus in *Hydrocleis* and *Limnocharis* could be spoken of as the retention of the ability of the chalazal megaspore to remain functional in part. These suggestions appear valid when Johri's (1938b) paper is consulted. Considering his Figs. 6 and 7, the former shows that the lower dyad cell has divided. The appearance in Fig. 7 suggests a state of functional and degenerating megaspores rather than primary micropylar and chalazal nuclei. Fig. 8 then shows an increase in the size, and what he interpreted as the three-nucleate stage could be thought of as the two-nucleate stage, with degenerating megaspores at both ends. The position of the vacuoles, or the lack of them, supports this reasoning. This argument can be followed through, but it has no particular advantage other than showing that derivations from the *Butomus* type (i.e. " Polygonum type ") of embryo sac are possible and that there are difficulties in referring to two megaspores (Johri, 1938b, Fig. 7) as the primary chalazal and micropylar nuclei.

In *Butomus* the development from the functional megaspore is quite straightforward and needs no additional explanation.

Holmgren (1913) reported that the upper and lower polar nuclei lie adjacent to one another for some time before fusing. This seems highly unlikely, as of all the sections observed, although polar nuclei were seen in various stages of migration, very few sections had the polars lying adjacent to one another. Nearly all mature embryo sacs seen had the " secondary " nucleus centrally suspended (Fig. 36). Although Holmgren made this statement, it was not substantiated by a drawing. No " striations " could be seen in the synergid cells as was reported by Holmgren. However, they were pointed at their micropylar end.

Contrary to Ward's (1880) belief, all antipodal nuclei are invariably present and have a linear arrangement. The cytoplasm between these nuclei is de-

limited by a thin wall or a plasma membrane.

It is interesting to note that the behaviour of the primary endosperm nucleus in *Butomus* and *Butomopsis* (Johri, 1936) is similar. In both cases the primary endosperm nucleus migrates to the chalazal end of the embryo sac and there divides to give the lower and upper endosperm nuclei which are cut off from one another. The lower endosperm nucleus behaves in the same manner in both genera. The entry of the pollen tube in the other three genera and its effect on the synergid nuclei is exactly the same as that described for *Butomus*.

The rôle of the "lower" endosperm nucleus is unknown. Its size and staining properties suggest activity in the early stages, but in later stages (Figs. 28, 29) it is degenerating. To say more than this would be only speculation. The membrane separating it from the cytoplasm above adopts a curvature which is the reverse of that shown by Holmgren (1913, Fig. 19).

Endosperm formation is definitely weak. By virtue of the first endosperm division this has been considered to be of the "Helobial type". At an early stage (Fig. 30) the proembryo apparently relies to a large degree on the basal cell to absorb material for its growth. Not only is the endosperm weakly developed but the basal cell by its size, shape and staining properties suggests activity. The nucellus also becomes depleted as growth of the embryo continues.

Finally, no irregularities or abnormalities such as Holmgren (1913) describes appeared in my material.

Summary

1. Numerous protuberances arise from the carpel walls before the microspore mother cells enter reduction.

No differentiation is observed in the developing ovules until the microspores have separated from their isobilateral tetrads and become embedded in the tapetal periplasmodium.

2. At this stage an archesporial cell becomes differentiated. It is covered by one or two rows of nucellar cells, usually by two rows.

3. The archesporial cell develops directly into the megaspore mother cell.

4. Two meiotic divisions result in the formation of a tetrad of megaspores. These megaspores are usually disposed in a " T-shape " arrangement. The linear arrangement sometimes observed seems to be derived from an archesporial cell covered by one row of nucellar cells.

5. The three uppermost megaspores degenerate. The lowest functions and by three successive divisions produces an eight-nucleate embryo sac with four nuclei at opposite ends.

6. These eight nuclei become organized into an egg apparatus (two synergid cells and one egg cell) and an upper polar nucleus at the micropylar end, and a lower polar nucleus and three antipodal cells (or nuclei) in linear arrangement at the chalazal end.

7. The polar nuclei migrate and fuse in the centre of the sac to form the " secondary " nucleus.

8. The pollen tube enters the embryo sac at the micropyle and during its passage in the embryo sac it passes through and destroys one synergid cell.

9. Double fertilization has been observed.

10. The primary endosperm nucleus migrates to the chalazal end of the embryo sac and divides once.

11. The lower endosperm nucleus divides no further and is separated from the upper endosperm nucleus by a plasma membrane, thus placing *Butomus* with others having a Helobial type of endosperm. The lower endosperm nucleus is extremely large and degenerates in later stages.

12. The upper endosperm nucleus migrates back to the centre of the embryo sac and remains suspended there in its cytoplasm while the zygote undergoes its first division to form the basal and terminal cells of the proembryo.

13. The upper endosperm nucleus undergoes a series of divisions resulting in about 30-40 endosperm nuclei.

Endosperm formation then ceases and the terminal cell of the proembryo undergoes several divisions which ultimately result in a straight embryo. The basal

cell is seen in many members of the Helobiales.

14. Attention is drawn to errors in Holmgren's account and it is now shown that:

(a) The archesporial cell does not cut off a tapetal cell, but enlarges to give rise to the megaspore mother cell directly.

(b) A membrane rather than a cell wall is formed between the upper and lower endosperm nuclei.

(c) The curvature of this membrane is the reverse of that depicted by Holmgren.

(d) Striations are not seen on synergid cell walls.

15. A suggestion is made that the type of embryo sac development in *Butomus* could have given rise to the quite different one reported for *Butomopsis*.

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THE UNISEXUAL FLOWER—A CRITICISM

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In his interesting " New Approach to the Problem of the Primitive Flower ", Dr. Sporne (1949) singles out twelve characters for correlation on a mathematical basis, and one of these is the unisexual nature of the flower. His figures, he considers, point strongly to the primitive flower being of this type. He is quite aware that a number of unisexual flowers are obviously derived from bisexual ones by reduction and if he had tried to eliminate these, his figures, he infers, would have been still more impressive towards regarding the flower as being primitively unisexual.

This is indeed perplexing in coming to an understanding of the evolution of the flower in all its aspects. I do not want in any way to challenge Dr. Sporne's mathematical calculations. They are apparently based on the statistical methods of Dr. Fisher — a pastmaster in this science. It would be instructive to see how the monocotyledons pan out by a similar mathematical approach. One hopes that Dr. Sporne will undertake this if he is not already doing so. His paper here criticized deals solely with the dicotyledons — the major group.

At the outset it is a little astonishing to find the Flacourtiaceae occupying the most primitive position, the Magnoliaceae and Anonaceae together taking the second place. His list of families is taken from Engler and Diels (1936). If he had adopted Hutchinson's system (1926) with its more restricted delimitation of families, the Magnoliaceae with the removal from it of such genera as *Drimys*, *Illicium*, *Schizandra*, etc., would then most likely have occupied the lowest place. It would not quite, but very nearly, have had in this Table I plus signs (+) for the whole of his supposed primitive characters, as a small section, *Kmeria* (Dandy,

1927.) composed of two closely allied species is distinguished by having unisexual flowers — doubtlessly derived from the bisexual condition characteristic of all the other numerous species of this family as restricted by Hutchinson.

In recent times the general trend has been to cast doubt on any existing angiospermous flower as being primitively unisexual, and one might add the same for fossil angiosperms as none of these so far discovered differs materially from any present day family.

The Amentiferae — a convenient term for linking together families possessing a catkin form of inflorescence — were long held, or at any rate some of them, as possessing flowers primitively unisexual; and Engler, commencing his classification of dicotyledons with such families, gave emphasis to this idea. Recent researches in my estimation all tend to cast doubt on this view — indeed they suggest that such flowers have had a bisexual ancestry. Sporne, in commenting on his figures for these catkin families, seems to me to take a somewhat illogical standpoint. To quote (p. 275): " It cannot be said, therefore, that catkin-bearing plants are primitive, as has sometimes been claimed, since some of them are relatively advanced... The group would appear to be an artificial one containing unrelated families, some advanced, like the Garryaceae, and representing a retrograde step from some bisexual ancestor, and *some quite primitive, like the Salicaceae, with flowers which are fundamentally unisexual* " (italics mine). Reading between the lines, perhaps, he would also include the Fagaceae as having primitive unisexual flowers. However, let us concentrate on the Salicaceae consisting of the two genera, *Populus* and *Salix*. Sporne has apparently overlooked or has not been convinced by Mary

Fisher's work (1928) on the flower of this family. Her investigations seem to me to leave little room for doubt that these flowers are greatly reduced, and that the remote ancestors of the Salicaceae had bisexual flowers with petaloid perianth and were most likely entomophilous. *Populus* with less reduced flowers is ane-mophilous; *Salix*, however, is largely entomophilous and suggestive that the willows have for the most part returned to insect pollination — a view put forward by Arber and Parkin (1907). Fisher supports this idea by pointing out that tropical species of *Salix* — clearly more primitive than temperate ones in floral structure — are wind-pollinated, and that their nectariferous-like scales are non-secretive. No one, I think, will now subscribe to the view put forward by Rendle (1903) that the disc or cup in the Salicaceae is the first indication of a perianth growing out of the floral axis.

Since the publication of my paper on classification (1945) in which recent work was stressed in favour of regarding the flowers of several catkin families as not primitively unisexual, an important paper by Moseley (1948) has appeared on the Casuarinaceae. The single genus, *Casuarina*, composing this isolated family has long been held as one of the most primitive of dicotyledons. Treub's classical work on this plant gave force to this idea. Thanks to Moseley's work, it looks as if the last stronghold of the Englerians will have to capitulate. He favours the derivation of *Casuarina* from the Hamamelidaceous complex. He goes into this view in all its main bearings and shows how his investigation on its wood anatomy fits in with such relationship expressed formerly by Bessey, Hallier and Hutchinson as gleaned from its floral morphology.

The upholders of the primitive nature of the unisexual flower may be inclined to point to some of the oldest geological formations containing fossil-remains of, say, the Salicaceae contemporaneous with those of the Magnoliaceae. This in itself does not prove that the flowers of the Salicaceae "are fundamentally unisexual". It can of course be maintained that some of the unisexual amentiferous families have nothing in common with the bisexual ones and have had a different origin; but this is tantamount to the acceptance of a diphyletic origin for the angiosperms as a whole. By doing so one is committed to recognizing the evolution of two independent groups of plants side by side with the same fundamental types, for instance, of stamen (microsporophyll), of carpel (megasporophyll), and of ovule with its stereotyped 8-nucleate embryo sac linked with triple fusion — surely stretching homoplasy to its limits !

How do those botanists, who favour the primitive angiospermous flower as being unisexual, derive the bisexual (hermaphrodite) one from it ? Wettstein did suggest some years ago that perhaps the magnolian type of flower was derived from a compressed inflorescence of unisexual flowers; but there is no evidence for this, and I am unaware of any botanist at the present day seriously upholding this idea. Sporne (p. 275) considers his unisexual primitive flower as polypetalous, i.e. having an indefinite number of free petals. The use of the term petal here seems a little too committal. Would not perianth member or even tepal have been preferable ? However, that is a minor criticism. Perhaps he will allow me to consider his primitive dicotyledon as having unisexual flowers of the two kinds each with a many-membered perianth. How from such a condition the bisexual stage was reached is not obvious — in fact puzzling. Shall we imagine a saltation which resulted in the female flower taking a leap as it were and landing on the top of the male, and shedding its perianth members in the process ?

This gymnastic jest brings me to a serious point in the morphology of the flower which does not seem to have been sufficiently stressed, viz. the invariable sequence of the essential organs. The stamens are always borne on the floral axis morphologically below the carpels. I say *morphologically*, for the inferior ovary when it occurs is generally held as derivative. It is usually due to the adhesion of the lower parts of the perianth to the ovary wall, or in some cases *may* have arisen through the flattening or hollowing out of the floral axis (receptacle) — a

debatable point and one I am rather loath to accept *in toto*.

If we regard the flower as originally unisexual at the angiospermous level, is it not a little strange in the evolution of the bisexual condition that no flowers have arisen with the sexual organs in the reverse position, viz. the stamens above the carpels on the floral axis or even arranged in a mixed order ? The invariable sequence these organs take in all bisexual flowers seems to me in itself to be a strong argument for the monophyletic origin of the angiosperms as a whole.

It can hardly be accounted for on physiological grounds since in lower groups of plants with bisexual cones (strobili) the reverse arrangement is the more usual. Among extinct plants the heterosporous cones of lycopods may be mentioned as having megasporophylls below the microsporophylls. Likewise, as far as is known this was the condition in the heterosporous Equisetales. Then this is, in the main, the rule but not invariably so in the large living genus *Selaginella*. Further it may be mentioned that in many unisexual monoecious angiosperms, when male and female flowers are borne on the same axis, the latter often occur below the former. This is well exemplified in the Monocotyledons, e.g. in the *Arum* family (Araceae), in *Typha* and in many species of *Carex*. In going through the families of the Dicotyledons with monoecious unisexual flowers this arrangement is often, though not always, the case. Even in the Compositae when the florets of the capitulum are not all bisexual, the outer ones (lower on the axis morphologically) tend to be female and the inner male.

The position of the stamens and carpels relative to one another in the bisexual angiospermous flower appears then to be exceptional; but not wholly so for the same sequence was followed in the fructification (anthostrobilus as coined for it by Arber and Parkin) of the extinct Bennettitales (Cycadeoidales) and also may be said to be present in the male flower of *Welwitschia* which bears at its apex a functionless ovule. On these grounds combined with other data Arber and Parkin (1908) were inclined to link these three groups, Angiospermae, Bennet-

titales and Gnetales, together as having branched off from some unknown ancestors which had evolved this type of cone.

Naturally I am not averse to regarding the remote heterosporous ancestors of flowering plants as having the two kinds of sporophylls borne apart, say, at the pteridospermous level, assuming for the time being the origin of the angiosperms from this plexus. The extinct pteridosperms are generally considered to have borne their sporophylls in a lax open condition. The Cycadophyta, which followed them and are generally held to have evolved from them, are distinguished by having their sporophylls for the most part collected together into cones (strobili). The few living representatives of what was perhaps a very extensive and varied group of plants in Mesozoic times have their sporophylls segregated into male and female cones. There is of course the well known exception shown by the genus *Cycas* itself, in which the microsporophylls only are in cones, the megasporophylls having apparently retained the primitive lax arrangement. The best known group of the fossil Cycadophyta are oddly enough the Bennettitales. The full elucidation of their fructifications by Wieland astonished the botanical world revealing what was nothing less than pteridospermous microspore-bearing fronds being caught up as it were into a strobilus with the essential organs in the angiospermous sequence.

Assuming the question as to how the two kinds of sporophylls borne separately on the same plant became associated together to form a strobilus is for lack of evidence merely a matter of surmise. The Bennettitales certainly act somewhat as a pointer as far as the microsporophyll is concerned, and perhaps the living *Cycas* may be considered so for the megasporophyll. The female part of the Bennettitean strobilus precludes any direct evolution from it of the angiospermous gynaecium.

We can visualize an early form of Cycadophyte — a Mesozoic pteridosperm one might term it — bearing in succession on the same stem (axis) first a series of one kind of sporophyll followed by a series of the other kind with perhaps an inter-

vening number of sterile fronds.[1]   What
induced the two kinds of sporophylls to
come together into close approximation
to form a cone after the angiospermous
fashion is of course a matter of specula-
tion.   We may assume that these early
monoecious forms with laxly arranged
sporophylls were wind-pollinated.   Insects
presumably were becoming abundant and
varied in early Mesozoic times and some
may have taken to feeding on pollen.
The plant as it were reacted to make use
of these visitations as a preferable method
of pollination to that by the wind.   Why a
bisporangiate (bisexual) cone arising in
this way took the anthostrobilate form
may have been the furtherance of cross-
fertilization and the prevention to some
extent of self-fertilization, assuming such
cones were originally borne vertically.
The insect might have used the apex of
the female part of the cone as a place of
alightment and then worked its way down
to the male part for the pollen.   The
secretion of nectar as a counter attraction
might have come later, though Robertson
(1904) in his interesting paper of some
years ago was inclined to give first place
to nectar as influencing the change-over
from anemophily to entomophily.

However, all this is largely imagination
and the origin of the flowering plants is
still very much " the abominable mys-
tery " as Darwin expressed it in a letter

written to Hooker in 1879.[2]   Its solution
or partial solution is not perhaps as
hopeless as some may think.   The rocks
are by no means fully explored as yet.
Hamshaw Thomas's discovery of the
Jurassic Caytoniales a few years ago is
heartening.   Though to my mind they
shed little light on the origin of the flower
as a whole, they may help in the elucida-
tion of the angiospermous ovule and its
wrappings.   Then it is not without the
bounds of possibility that some living
plant may yet be discovered to help in
the solution.   Just recently what was
known hitherto as a fossil conifer was dis-
covered as still in existence and given the
cumbersome name of *Metasequoia glypto-
stroboides*.

Summary

Reasons are given for considering all
unisexual flowers of angiosperms as deri-
vative.   All are held to have had bisexual
(hermaphrodite) ancestors.

The unisexual catkin-bearing families,
long held as primitive, would appear to
have had for the most part their origin
from a bisexual Hamamelidaceous plexus.

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1. *Isoetes* is interesting in this connection as
affording an example of the two kinds of
sporophylls following each other on the same
axis, but in the reverse sequence to that occur-
ring in the Angiosperms.   First megasporophylls
then microsporophylls are developed and finally
sterile foliar organs.

2. In " More Letters of Charles Darwin ",
Vol. II, London, 1903, p. 20.   The full sentence
runs as follows: " The rapid development as far
as we can judge of all the higher plants within
recent geological times is an abominable mys-
tery."

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RELATIONSHIPS OF THE EPHEDRALES

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Introduction

The " Gnetales ", as this term has been commonly used, comprise a small group of plants — *Gnetum*, *Welwitschia*, and *Ephedra* — prominent because they have seemed to form a connecting link between gymnosperms and angiosperms, and because of their extraordinary morphology and diverse habit.   They are gymnosperm-like in that their ovules are naked and borne in cones, and angiosperm-like in that the ovules and microsporangia are borne on somewhat flower-like fertile shoots and their xylem possesses vessels.   The three genera were long considered to constitute the single family of the order, the Gnetaceae, although it was evident that the family was strongly heterogeneous.   As early as 1874, Bertrand, in an anatomical study of the Gnetaceae remarkable for its extent and thoroughness, stated that the three genera " forment des groupes absolument séparés ".   In gross structure the genera are remarkably unlike; in critical morphological characters they are only loosely held together.   The heterogeneous nature of the order has become increasingly apparent in recent years and has been recognized by a constantly changing taxonomic treatment in which the group has been split, first into two families — in two ways, with different groupings of the genera — and then into three; later the families were grouped in two orders, more recently in three.

Morphological evidence strongly supports a break-up of the old order, with the setting up of three families (Ephedraceae, Welwitschiaceae, Gnetaceae) and the establishment of three orders (Ephedrales, Welwitschiales, and Gnetales *sensu stricto*), each to contain one family.   Further, morphological evidence indicates that the Ephedrales have no phylogenetic relationship to the Welwitschiales and the (new) restricted Gnetales. Pearson (1931) probably first suggested ordinal rank for the Ephedraceae, having in 1929 questioned whether this was not justified.

Morphological studies of the Gnetales and views as to the relationships of the group were, even in 1912, " innumerable"; Lignier and Tison (1912) stated that the little group " ont provoqué le plus de recherches et ont le plus exercé la sagacité des botanistes ".   At that time Lignier and Tison, and again, in 1929, Pearson, summarized and discussed the morphological literature in monographic treatments.   Since 1929 rather few papers dealing with the group have been published and another paper dealing broadly with the same genera may seem unnecessary. But the trend in taxonomic treatment in recent years has been to emphasize differences rather than similarities in the genera, and the recent re-interpretation of the cordaite cone—that the ovule in *Cordaianthus* is appendicular and not cauline (Schoute, 1925; Florin, 1939) — makes necessary a new comparison of the cordaites

and the Gnetales, especially *Ephedra*, which in the earlier years was occasionally considered to resemble the cordaites (Bertrand, 1878; Saporta & Marion, 1885; Solms-Laubach, 1887; Thibout, 1896).

Underlying the morphological support for the present treatment are advances in the field of morphology in recent years: the acquisition of much new information concerning the morphology of vascular plants, especially of extinct groups; a better understanding of the part played by parallel and convergent evolution; and the recognition of the importance of anatomical evidence in the solution of problems of structural reduction.

The morphological nature of the cones of the cordaites and conifers is now better understood and provides a new basis of comparison for the interpretation of the cones of the gnetalean genera. The possession of vessels as a significant character in phylogenetic relationships is now recognized as valueless. (Vessels have arisen independently in the Selaginellaceae, Polypodiaceae, Gnetales, and angiosperms — in the last group undoubtedly several times — and most ferns and some primitive angiosperms lack them.)

On a morphological basis the break-up of the old family Gnetaceae apparently began in 1909 when Pearson pointed out that *Ephedra* should be set apart from the other genera. This suggestion has steadily gained ground both morphologically and taxonomically.

That the Ephedraceae stand much further from the Welwitschiaceae and the Gnetaceae than the last two families do from one another has been suggested in several studies, both taxonomic and morphological, and has recently received strong support from Florin (1931, 1934) who pointed out that the stomatal apparatus of *Ephedra* belongs to a primitive basic type different from that of *Welwitschia* and *Gnetum*. That the Ephedraceae are not even distantly related to the Welwitschiaceae and Gnetaceae is also shown by differences between *Ephedra* and the other two genera in fundamental structure of the cones (especially ovule position), in nodal anatomy, in primary structure of the stem, and in wood structure. Most important in the establishment

of a major difference in cone structure is the demonstration of strict homology in the microsporangiate and ovulate cones of *Ephedra*. If the two kinds of fertile shoots are homologous — as the evidence here presented seems to demonstrate — the ovule is terminal on a lateral appendage of the fertile shoot, as are the microsporangia, and not cauline (terminal on the shoot axis), as it has been usually considered to be and as it apparently is in *Gnetum* and *Welwitschia*. So · important a morphological difference in itself indicates a great phyletic gap between the Ephedraceae on the one hand and the Gnetaceae and Welwitschiaceae on the other.

Recognition of ovule position in the Ephedraceae as appendicular not only indicates the wide separation of this family from the Welwitschiaceae and Gnetaceae but makes necessary acceptance of ordinal rank for the group. Further, the reevaluation of the morphology of the fertile shoots of *Ephedra* and comparison with the cones of the cordaites, as recently reinterpreted by Schoute (1925) and Florin (1939), suggest relationship of the Ephedrales to cordaite stock or to an ancestral stock common to cordaites and conifers.

Terminology

To make clear the homologies between the reproductive structures of the cordaites, conifers, and *Ephedra*, it is necessary to describe briefly these structures in *Ephedra* because of the confused and misleading "angiosperm-centred" terminology almost universally used in descriptions of this genus. Because of the view now gaining acceptance that the Gnetales are not in any way phylogenetically related to the angiosperms, such terms as inflorescence, flower, perianth, stamen, anther, antherophore, filament, and column — as applied to the Gnetales — should be discarded. The implications of their use are most unfortunate. The terms came into use partly because of superficial resemblance of some of the reproductive structures to those of angiosperms and partly because the group seemed to provide a transition from conifers to angiosperms. In this paper the terms used are those believed morphologically

suitable, those that suggest certain or probable homology with organs in other gymnosperms, especially those groups that seem closest to the Gnetales, the conifers and the cordaites.

The so-called *cones* or *strobili* of *Ephedra* are morphologically equivalent to the ovulate cones of conifers and these terms are suitable and should be retained for *Ephedra*. The cluster of cones, sometimes unfortunately called an " inflorescence ", is best called a *cone cluster*. The cones themselves have also frequently been called " inflorescences " on the basis that they consist of an axis with " flowers " subtended by bracts. The *Ephedra* cone, both ovulate and microsporangiate, is compound because fertile shoots are present in the axils of the bract-like appendages of the main axis. These fertile shoots, commonly called "flowers", are best called simply *fertile shoots*; no other suitable term exists. The *bracts* subtending the fertile shoots are homologous with those of conifers subtending the axillary fertile short-shoots of the ovulate cone; the term is applicable in both groups and also to the homologous organ subtending the cone-like *fertile shoot* of the cordaites. The sterile appendages of the fertile shoot of *Ephedra* are here termed *bracteoles* since they are small, bract-like appendages of a secondary axis. In the microsporangiate shoot they have usually been called the " perianth " of the " flower ".

The " stamen " of *Ephedra* is the *microsporophyll* or pair of microsporophylls in this treatment, its " anthers ", the *microsporangia*. The " column " consists of two fused microsporophylls and is not the tip of the fertile-shoot axis; it may be called a column, if morphological value is not suggested. The term " filaments " is sometimes applied to the microsporophylls and sometimes to the stalks of the microsporangia. (In one interpretation, fusion of these stalks forms the column.) The *megasporophyll* is nameless in the older literature because its presence was not recognized; it exists either as a vestige below the ovule, forming a base on which the ovule sits, or, more likely, has been wholly lost. In the ovulate shoot the pair of connate bracteoles constitute the

so-called outer integument. The term integument, as it has been applied to these bracteoles, is most unfortunate because of the suggestion that it is homologous with the outer integument of angiosperms. Its loose, husk-like nature and obvious fused-bracteole origin should have prevented this interpretation as an integument, but *Ephedra*, with two integuments, fell into line with *Gnetum*, with " three ", and *Welwitschia*, with " two ". The criticism of Lam (1948) and others of the reading of angiosperm morphology downward into the gymnosperms is well justified here.

The character that perhaps served chiefly in support of the angiosperm-like nature of the Gnetales was the supposed bisexual structure of the cones of the three genera. But uncritical interpretation underlay this, for, though the fertile shoot of *Welwitschia* is doubtless bisexual and that of *Gnetum* is perhaps so, that of *Ephedra* is monosporangiate. Mehra (1950) has described " hermaphrodite flowers " in one species, but these seem mere monstrosities. " Bisexual cones " have indeed been described in *Ephedra* (Thibout, 1896; Takhtadjan, 1950 ; and many others) and the *cones* of this genus are indeed sometimes bisexual, but in the other two genera this character is possessed, not by the *cone*, but by the *fertile shoot*. The strictly unisexual nature of the fertile shoot in *Ephedra* accentuates the wide separation of the Ephedrales from the Welwitschiales and Gnetales and strongly supports ordinal rank for the Ephedrales.

Discussion

The significant points in the newer understanding of the cordaite cone are that the ovule is borne terminally on an appendage, not terminally on an axillary shoot, as has usually been believed, and that the ovulate cone is therefore simple, not compound. This brings the ovulate cone into line with the microsporangiate cone which is simple, with the sporangia borne terminally on appendages.

The ovule of *Ephedra*, like that of *Cordaianthus zeilleri*, has been considered terminal on the axis of the fertile shoot (" flower "). And the microsporangia of *Ephedra* also have been considered

terminal on the axis, or column — " a projecting axis that bears two or more sporangia "; a " stamen . . . evidently an axile structure " (Coulter & Chamberlain, 1910, p. 371). (The microsporangia of *Cordaianthus* were recognized as terminal on appendages.)

In both *Cordaianthus* and *Ephedra* the position of the ovule has been similarly misinterpreted: called terminal on a lateral axis, when it is really terminal on an appendage or sporophyll. In both genera, evidence of its true position has indeed been obscure, but it is surprising that in *Ephedra* the appendicular position has not been recognized during the numerous morphological studies of the genus. Several authors have suggested that it might be appendicular. Bertrand (1878), Van Tieghem (1884, 1891), Thibout (1896), Sykes (1911), Lignier and Tison (1911a), Church, (1914) Emberger (1944), and a few, like Parkin (1922), have claimed it to be so. In past years there has been failure to recognize the extent of apparent changes in organ position that take place under extreme reduction, for example, in pseudo-terminal carpels and falsely basal ovules in angiosperms, and in falsely axillary ovules in conifers, as in *Pherosphaera* (where the fertile shoot is lost and the ovule is sessile in the bract axil). Such changes under reduction were discussed by Parkin (1922) who declared the ovule position in *Ephedra* to be pseudo-terminal and acquired under great reduction.

Failure to recognize apparent position of an organ as false results from the common practice of studying a single member of a group as representing that group, rather than from the comparative study of all members of the group. And today Lam (1948, 1951), in advocating the practice of accepting structure *as it is* — the " typological " method — instead of looking for underlying fundamental structure as shown by comparative study and anatomical evidence, is similarly making false interpretations.

The interpretation of ovule and sporangium position in *Ephedra* as terminal on the axis could hardly have arisen if the entire genus had been considered comparatively, because the species generally recognized by taxonomists as primitive,

when all characters are considered, have no column but, in its place, a pair of distal appendages (microsporophylls) bearing terminal sporangia (Fig. 1, A, B). And — most important — in a few species the vestigial tip of the fertile-shoot axis projects between these appendages for a short distance (Fig. 1, A). The presence of this true axis tip in itself shows the interpretation of the column as axial to be false. Most species, however, show the pair of opposite fertile appendages connate adaxially (Fig. 1, C, D)[1]. The fusion of these two appendages ventrally forms a median columnar structure (Fig. 1, D), a pseudo-axis tip, which has unfortunately often been considered the true continuation of the axis. Under this false interpretation the microsporangia appear terminal and cauline, and the position of the ovule was therefore readily considered the same, especially when superficially it seemed to be so. Or perhaps the apparently terminal, cauline position of the ovule led to the interpretation of the column as axis, and the position of the microsporangia therefore the same as that of the ovule.

The opinion that the microsporangiate and ovulate fertile shoots are homologous is by no means new; it has been held by several students of the genus, but the full implication of this interpretation seems not to have been recognized. Pearson (1929) states that discussion of the homology of the fertile shoots ceased as the opinion grew that the unisexual flowers of *Ephedra* were derived by reduction from bisexual flowers such as those of *Gnetum*

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1. A similar median " column " suggesting an axis is formed in the orchids by the adaxial adnation of style and filaments, and in the willows by connation of stamens in two-stamen species. The orchid column was misinterpreted until its nature was demonstrated by Darwin (1899) on the basis of anatomy. Lam (1948) and van der Hammen (1948) today fail to recognize the true nature of the connate stamens in *Salix*, which is also demonstrable by anatomy and by comparison with other species of the genus (Fisher, 1928; Eames, 1951). Other examples of obvious connation of organs on opposite sides of an axis tip are those of ovaries of *Mitchella* and species of *Lonicera* where flowers in the axils of opposite leaves fuse by their ovaries above the stem tip, forming a two-flower ovary that seems terminal. The two leaflets in *Bauhinea* are similarly connate above and beyond the rachis tip.

and *Welwitschia*. *Ephedra* fell in line with its " sister genera ".

In *E. altissima* and *E. trifurca*, and some other uniovulate species, the one ovule is apparently terminal on the cone axis. This condition has naturally been considered morphologically important — a " unique ovule " (Strasburger, 1872) — but the ovule in this position has been shown by Thoday and Berridge (1912) for *E. altissima*, and by Florin (1934) for *E. fedtschenkoae*, to be morphologically double. The two ovules in the axils of the distal bracts are connate over the cone-axis tip. The fusion is partly ontogenetic and partly phylogenetic. All stages are found. Lignier (1903) earlier had stated that the solitary ovule in *E. altissima* is truly axile, its apparently terminal position resulting from the abortion of one of the upper axillary pair. (This is essentially what Thoday and Berridge have shown.) A more common condition in uniovulate species is that where one of the distal pair of ovules aborts without fusion with the other ovule and remains small, and the normal ovule crowds into a false terminal position. Ovules apparently terminal on the cone are morphologically axillary and are not significant in the morphology of *Ephedra*.

Morphology and Anatomy of the Cones of Ephedra

The general nature of the reproductive structures of *Ephedra* is well known to morphologists and taxonomists, but the following description summarizes the interpretations supported in this paper.

The cones consist of several decussate pairs of bracts borne on short axes, forming ovoid or subspherical structures. In the

FIG. 1 — Diagrams of fertile shoots of *Ephedra* showing progressive stages in evolutionary modification: *A-D*, microsporangiate, based on living species; *E-I*, ovulate, hypothetical, based on anatomy and comparisons with microsporangiate shoot. In the longitudinal diagrams the sporophylls are shown, for convenience, as though anterior-posterior, though actually lateral as shown in transverse diagrams.

axils of part of the bracts — in most of them in microsporangiate cones, in one to three uppermost pairs in ovulate cones — are borne slender, fertile axes, each with a pair of bracteoles. The bracteoles, free or connate by their margins, stand anterior-posterior on the axis (Fig. 1). They are prominent in microsporangiate cones but in ovulate cones are obscure and may appear to be absent until the cones are critically studied. The ovulate fertile shoot can best be interpreted by detailed comparison with the microsporangiate shoot. In spite of superficial lack of resemblance (the ovulate fertile shoot has often been described as an " axillary ovule "), the external and internal structure show the two types of shoot to be strictly homologous throughout. The microsporangiate shoot bears two microsporophylls on its axis just above the bracteoles. In most species these sporophylls are ventrally connate above the axis tip, forming the " column " (Fig. 1, C, D). In the more primitive species the microsporophylls are free and the axis tip may project beyond their bases (Fig. 1, A, B). Emberger (1944), basing his statements on Hagerup (1934), describes the fertile shoot as having, above the bracteoles, " a third leaf, the stamen, more or less branched by dedoublement [the two sporophylls], covering almost completely the tip of the axis [the fused sporophyll bases nearly cover the axis tip ?] and a fourth sterile leaf [the axis tip itself ?]. The first two and the fourth constitute the perianth." According to Hagerup's and Emberger's understanding of this " flower ", therefore, one of the perianth parts stands above the stamen ! No student of the group but Hagerup seems to have seen the " fourth leaf ", unless to recognize it as the axis tip. The interpretations of Hagerup and Emberger are obviously inaccurate.

The ovulate shoot has been greatly telescoped and reduced. The axis has been shortened both above and below the bracteoles. The bracteoles stand almost at the base of the axis and the ovule is sunken between the bracteoles (Fig. 1, I). The connate bracteoles are loosely appressed to the ovule and form a husk-like envelope which has been commonly called the outer integument.

The anatomical proof of these interpretations is presented in Figs. 1-4. Fig. 1, A-D shows diagrammatically in longitudinal section and horizontal plan the various types of microsporangiate fertile shoot of living species. (In all longitudinal diagrams of this figure the sporophylls are shown, for convenience, as though anterior-posterior, like the bracteoles, though they are lateral as shown in the horizontal diagrams.) Fig. 1, A, B shows the structure of primitive species, the sporophylls free and surrounding the axis tip. Fig. 1, C, D shows the sporophylls fused adaxially to each other, in D, through their entire length.

Fig. 2, A shows on a larger scale the shoot shown in Fig. 1, A, B, with the course of vascular bundles indicated by dotted lines. (The axis is greatly shortened and the sporophylls are shown as anterior-posterior, as in Fig. 1.) The tip of the axis is shown with a dotted line to indicate that it is not present in all species with free sporophylls. Fig. 3, A shows cross-section diagrams at levels indicated in Fig. 2, A. The bract has two traces which arise from the cone-axis stele well apart, as do traces to the leaf. These traces continue to the top of the bract, one near each side (Fig. 2, A). In Fig. 3, A, a-a are seen the three bundles that supply the fertile-shoot axis. From these are given off to the posterior bracteole one (median) or two (lateral) weak traces (Figs. 2, A ; 3, A, b-b, c-c) that extend as weak bundles for various distances in the bracteole. The anterior bracteole typically has no vascular supply but may have one weak median bundle. In some species neither bracteole has any vascular supply (Strasburger, 1872; Pearson, 1929; Goebel, 1933). Above the bracteoles the three stelar bundles fuse to form one large flat bundle (Fig. 3, A, b-b). The large bundle then splits into three small bundles. The two lateral bundles constitute traces to the two sporophylls, one entering each sporophyll base when the sporophylls are free, and both entering the column side by side when the sporophylls are fused. The median bundle continues into the free axis tip if this is present; if the axis does not extend beyond the sporophyll bases, the bundle dies out between or below them as the

vestigial tip of the stele (Fig. 2, A). Within each sporophyll the single bundle forks dichotomously once or twice (Fig. 4), a single bundle tip extending into the septum of each sporangium. The existence in some primitive species of the axis tip with its vascular supply, and in other species of a vascular remnant below the position of this tip when the tip is lacking, is proof that the sporophylls are lateral organs, and that the interpretation of the column as an axis is erroneous. The vascular anatomy of the typical column — two bundles which arise from opposite sides of the stele — is further proof of the appendicular nature of the column. In ontogeny, the column, whether forked or simple, arises from two separate primordia — further evidence of its double nature. Goebel (1933) states, surprisingly, that whether the column is axial in nature or consists of fused sporophylls "scheint mir

nicht von Bedeutung", but surely whether the sporangia and the ovule are cauline or not is most important.

The interpretation of the condition where the sporophylls are free or only partly fused as a forking of the axis is the result of reading the series in microsporophyll form throughout the genus in the wrong direction. Types with free sporophylls (Fig. 1, A, B) are found in species recognized by taxonomists as primitive, especially E. distachya and E. intermedia, but occur occasionally in many species that have the higher numbers of microsporangia.

Fig. 3, B shows the anatomical structure of the typical ovulate shoot, that with a more or less triangular ovule, and that with an ellipsoid ovule. The ellipsoid ovule usually is a double one, one that appears to be terminal on a cone, as in E. altissima and E. trifurca. Anatomically
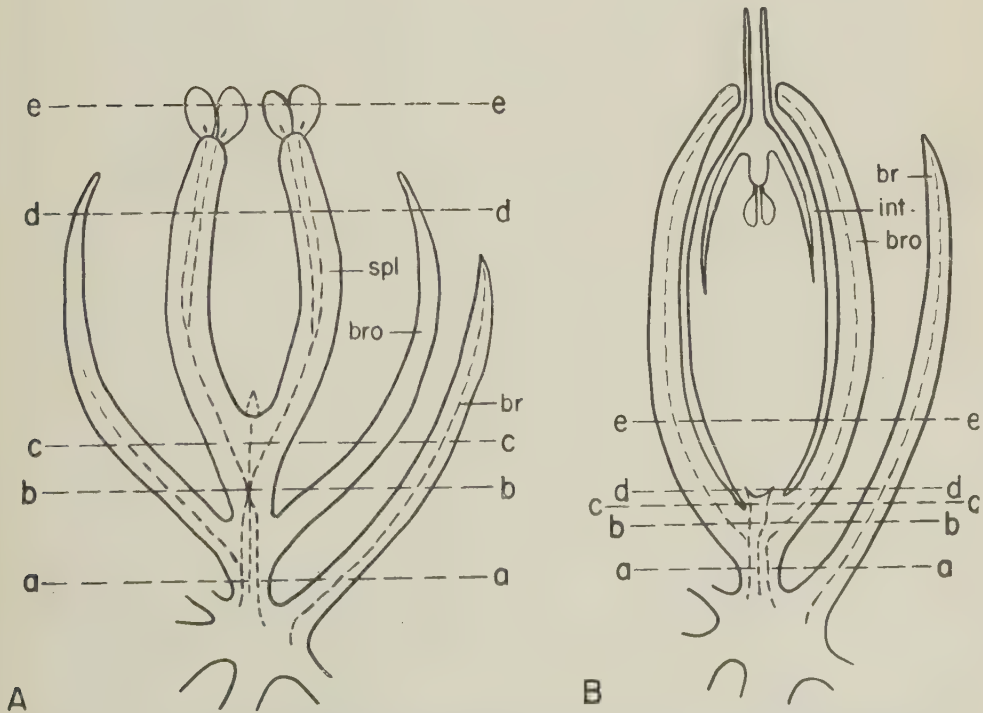

Fig. 2 — Longitudinal diagrams of ferrtile shoots of Ephedra, the sporophylls shown for convenience as though anterior-posterior: A, microsporangiate; B, ovulate. Vascular bundles shown by dotted lines. Cross-sectional levels a-a to e-e are those illustrated in Fig. 3. br, bract; bro, bracteole; int, integument; spl, sporophyll.

these ovules are different because the terminal ovule is double at least in its sheath. The typical triangular ovule has two bundles in the lateral angles of the sheath, with sometimes a third in the weaker anterior angle; the double ovule has four or six in its sheath. Where abortion of one ovule has allowed the opposite ovule to become apparently terminal, the surviving ovule may be ellipsoidal, but has the anatomy of a triangular ovule. The long confusion over the number of bundles in the " outer integument "— 2, 3, 4 or 6 — is explained simply on this basis. The fertile shoot trace supply is the same as that of the microsporangiate shoot — three independent strands, one anterior, the others
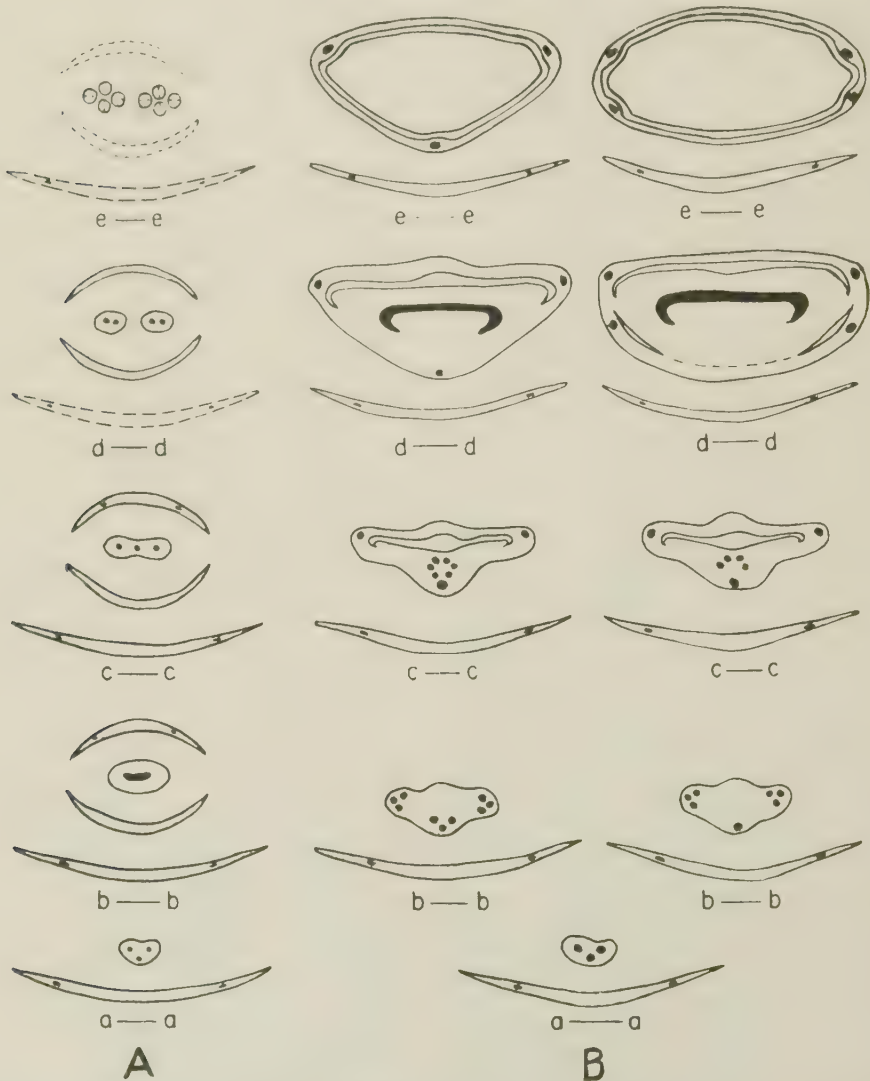

FIG. 3 — Series of cross-sectional diagrams of fertile shoots of *Ephedra* at levels indicated in Fig. 2. Vascular bundles in solid black. *A*, microsporangiate; *B*, ovulate shoot, showing two types of structure, the right-hand series of morphologically double ovules with vascular supply of two " outer integuments ", so-called.

lateral (Fig. 3, *B, a-a*).   In the short base of the shoot, which becomes flattened, all three of these or only the two lateral bundles (Fig. 3, *B, b-b*) fork almost at once into three similar strands.   Of each group of three so formed, the lateral members (6 or 4) move toward the centre of the shoot axis and form a stele-like cluster (Fig. 3, *B, c-c*).   Fusion of the six or four bundles results in the formation of a large bundle, crescent-shaped in cross section, open anteriorly (Fig. 3, *B, d-d*). This large strand becomes half-cup-shaped in the base of the ovule proper for which it constitutes the vascular supply.   The position and form of the vascular supply of the ovule suggest that the ovule is lateral, not median, in position.

In both the older and more recent literature the inner integument of *Ephedra* has been reported to show evidence of consisting of two fused organs. This evidence consists of the origin of the integument in some species from two primordia and of the presence in it of a vascular supply of two bundles.   In other species the integument is reported to arise as a ring, and in only two or three species has a vascular supply been described.   Unfortunately little is known about the detailed development and structure of the young ovule.   The vascular system of the mature
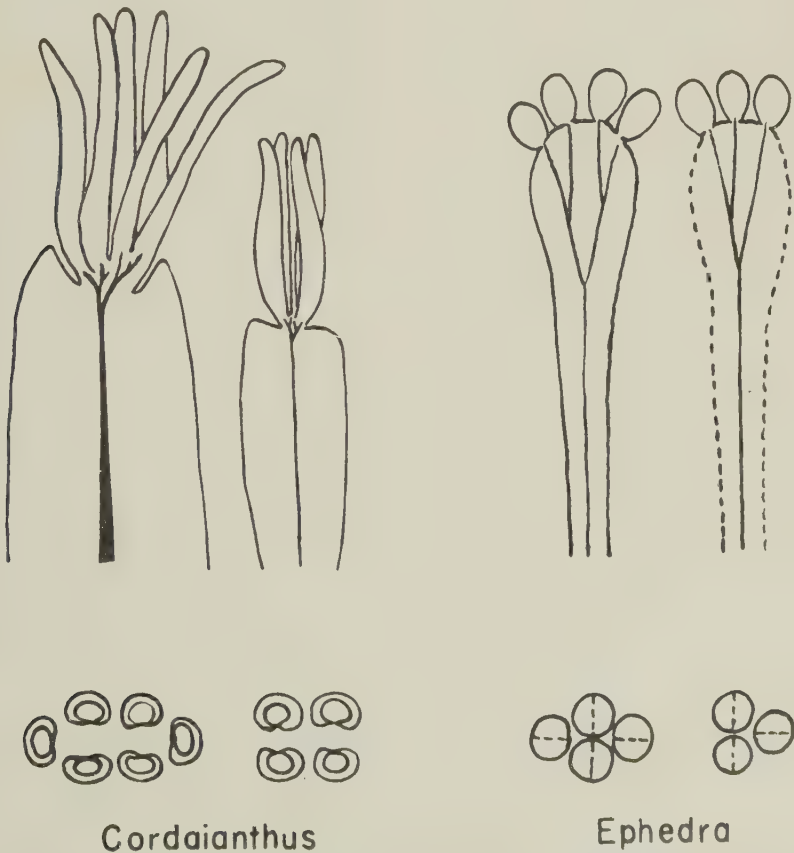

Cordaianthus   Ephedra

F1G. 4 — Diagrams of microsporophylls of *Cordaianthus* and *Ephedra* showing similarities in form and anatomy, and in position, number and arrangement of sporangia.   Dotted outline of sporophyll indicates fusion with a similar opposite sporophyll in the column.   (*Cordaianthus* after Florin.)

ovule is described and figured as consisting of two systems: a lower, supplying the bracteolar sheath, and an upper, supplying the inner integument. Published figures usually show the upper system ending in the base of the integument but it is said to consist of two bundles in *E. trifurca* (Land, 1907) and *E. americana* (DeHaan, 1920) and in the second species to extend as far as the level of separation of integument and nucellus. The location of these two bundles—an important point—seems not to have been described. This evidence from ontogeny and vascular anatomy — that the (" inner ") integument consists of two organs — provides important additional evidence of the relationship of *Ephedra* to the cordaite stock. The seeds of the cordaites are flattened anterior-posteriorly and show on their faces median longitudinal lines of apparent fusion of two organs which constitute the integument, and the anatomy of the integument shows two lateral bundles.

The two bundles remaining in the margins of the flattened fertile-shoot axis (Fig. 3, *B, c-c*) form the two traces of a posterior bracteole (Fig. 3, *B, d-d*), which becomes free at this level, somewhat before the anterior bracteole does, because the anterior bracteole is slightly adnate to the base of the ovule. The bundle remaining on the anterior side — whether it is an original bundle of the shoot axis or remains from a division into three — forms the single weak trace of an anterior bracteole, or dies out. (In both microsporangiate and ovulate fertile shoots the anterior bracteole is more reduced than the posterior. In other plant groups also, where an axillary shoot is reduced, there is greater adnation and reduction on the anterior than on the posterior side of the shoot.) In microsporangiate shoots the anterior bracteole has either no vascular supply or one median bundle, whereas the posterior bracteole has one or two bundles; in the ovulate shoot, similarly, the anterior bracteole has one or none and the posterior has two bundles.

From anatomical evidence the homology of the microsporangiate and ovulate shoots is complete. It is obvious that the " second integument " represents the anterior-posterior pair of bracteoles of the fertile shoot, connate and loosely investing the ovule. This interpretation has been held by many authors (Hooker, 1863; Parlatore, 1868; Strasburger, 1872; Bertrand, 1878; Worsdell, 1901; Lignier, 1903; and many others), but anatomical support for it has not been presented. The apparent presence of extra integuments in *Gnetum* and *Welwitschia* directed attention away from the real nature of a similar structure in *Ephedra*. Strasburger's (1872) excellent illustrations of ontogeny and accompanying data indicated that the outer integument consists of two anterior-posterior bracts. This was also Goebel's (1933) final opinion. Land (1904) held from ontogenetic evidence that four organs made up the outer integument, but Land studied *E. trifurca* in which the ovule is of double nature, with the outer integuments of two ovules (four bracteoles). DeHaan claims several bracts are present in this integument but does not support his view with evidence. Hagerup (1933) stands alone in denying the presence of even a vestige of the anterior bracteole. He interprets the posterior bracteole as two fused laterals, using to support his view what appear to be oblique sections. He considers the bracteoles in both kinds of fertile shoots alternate (his figures of developing shoots are apparently from oblique transverse sections), but external form and vascular structure show them always opposite as are all bracts and leaves in the genus. He believes the outer integument to be the megasporophyll but gives little or no supporting evidence.

The axis of the ovulate fertile shoot is very short but its presence is clear (1) in the origin of its stele from three bundles exactly as in the microsporangiate shoot and a vegetative axillary shoot; (2) in the giving off of traces to the bracteoles as in the microsporangiate shoot; (3) and in the formation of a well-marked stele above the bracteole trace origin. In the advanced cordaite, *Cordaianthus zeilleri*, the ovule-bearing sporophyll is similarly reduced but present as a short flat stalk. The reduction of the fertile shoot and sporophyll in *Ephedra* is indeed extreme, but not more so than that of the ovulate short shoot (" fertile scale ") of the highly specialized and reduced conifer, *Pherosphaera*. In

this genus also proof that the ovule is not axillary and sessile lies in homologies with less primitive conifers. (A vegetative short shoot reduced to a mere base with one psudo-terminal appendage is seen in *Pinus monophylla*.)

Comparison of the microsporangiate and ovulate fertile shoots shows the same gross structure in both, with much greater reduction in the ovulate. In both, the anterior bracteole is more reduced. In both, the two sporophylls are reduced by intimate fusion, in the microsporangiate shoot to what appears to be one (in the majority of species) accompanied in some species by reduction of sporangia to one per sporophyll; in the ovulate shoot to a mere base for the terminal ovule of one sporophyll, or to complete loss. Reduction of sporangia to one per sporophyll has taken place in both types of fertile shoot.

Homology of the cones and of the fertile shoots suggests that there were originally two ovulate sporophylls per fertile shoot and that one has been lost, the other reduced to a mere ovular base or lost entirely. Theoretical stages in such a phylogenetic change are indicated in Fig. 1, *E-I*: the long axis progressively shortened both above and below the bracteoles; one sporophyll becoming sterile, then lost; the surviving ovule gradually enclosed within the connate bracteoles, which became appressed to it; and with shortening of the fertile-shoot axis and enclosure of the ovule within the bracts, the micropylar tip of the true integument becoming elongated, projecting beyond the enclosing bracteoles and the large subtending bract, which would exclude pollen.

In some species, following, or in part preceding, fertilization, the apex of the gametophyte grows upward into the floor of the pollen chamber, lifting the nucellus and even filling the chamber, (Land 1907; Maheshwari, 1935; Khan, 1943). The projection so formed has been likened to the " tent-pole " of the cordaites and *Ginkgo*. If this is a vestigial " tent-pole ", as seems probable (a pointed apex is present in some conifers also), the structure adds further evidence for the relationship of *Ephedra* and the cordaite stock, especially since the cycadophyte line has no such structure.

The phylogenetic history of the microsporangiate fertile shoot is preserved in the living species. Several stages in fusion and reduction are shown diagrammatically in Fig. 1, *A-D*. The bracteoles were not reduced and remained free or somewhat connate. Connation of the sporophylls progressed distally, the flattened sporophylls narrowing until a pseudo-terminal columnar structure was formed. As the sporophylls fused, the axis tip between them (still present in a few species) was lost, though its vascular tip remains in vestigial form in some species beyond the point of departure of the bracteole traces (Figs. 2, *A* ; 3, *A*, *c-c*). The sporophylls remain elongate, their tips with the sporangia projecting at pollen shedding well beyond the enclosing bracteoles and bracts. The number of sporangia, eight in the primitive species, is reduced to one or two per sporophyll in the most specialized species.

The two-chambered sporangia are commonly described as synangia but the vascular supply extends into the base of the partition wall, evidence that the structure is probably a chambered sporangium, not a synangium.

The demonstration of complete homology of the fertile shoots seems to make certain the appendicular position of the ovule. A great variety of opinions as to the position of the ovule has been held, but that of the majority has been that it is cauline. Among those who have believed it to be appendicular are Van Tieghem (1869, 1872, 1891), Bertrand (1878), Lignier (1903), Lignier and Tison (1911, 1912), Thoday and Berridge (1912), Sahni (1921); and Parkin (1922). Saporta and Marion (1885) maintained that "L'ovule y a ténu la place d'une feuille dont il n'est rien reste . . . il est dévenu finalement axile et terminal". Lignier and Tison (1911) say that the ovule " semble prolonger l'axe floral, mais est très problement foliaire ". Thoday, in an editorial comment in Pearson (1929), remarks that Church (1914) states that under xerophytic stress the function of the megasporophyll soon vanishes " leaving its limiting residual ovule alone in its place " as in the Gnetales. Parkin (1922) probably first critically discussed the ovule position as not truly

terminal, stating that its position is pseudo-terminal as a result of reduction. He likened the morphological condition to that of the basal — often so-called terminal — ovule in certain angiosperms. Emberger (1944) indirectly, and perhaps inadvertently, also calls the ovule position foliar, though apparently basing all his interpretations for this genus on the studies of Hagerup (1934, 1936, 1938), who considers the nucellus cauline. Sykes (1911) suggested that " although the ovules are now in an axillary position, they may not originally have been cauline ".

As to the position of the ovule on the fertile leaf there have been various opinions, but among these there is apparently none that the position is terminal, which is surprising since the microsporangia are terminal. Van Tieghem (1872, 1891) maintained that the tip of the axis is aborted and the ovule borne on the " dorsal " face of one of the bracteoles constituting the outer integument. Lignier and Tison (1911) suggested the same position — on one of the " carpelles de l'ovaire ". Sykes (1910) placed the ovule on the subtending bract. Lignier and Tison at first (1911) considered the fertile leaf as having the aspect of an axis by secondary modification. (The anatomy of this axis-like base clearly shows that it is an axis and not a modified appendage.) These authors later (1912) state that the fertile leaf " a totalement disparu, ne survivant que par son ovule ". In this opinion, the writer concurs. In the conifers several genera (*Podocarpus, Dacrydium, Acmopyle, Microcachrys, Pherosphaera*) form a series of similar but more extensive reduction in which an entire fertile shoot is reduced to a base, and in its extreme form (*Pherosphaera*) to the ovule alone.

Comparison of Cones of Ephedra with those of other Gymnosperms

Since the ovule of *Ephedra* is borne on an appendage and is not cauline, *Gnetum* and *Welwitschia*, with ovules apparently cauline, need no longer be considered related to *Ephedra*. The resemblances of *Ephedra* to other gymnosperms, when all features, vegetative and reproductive, are considered, are closest to the cordaites and conifers, especially the cordaites. The strongest resemblances are in general cone structure, anatomy of sporophylls, and position of microsporangia and ovule. In addition to these are similarities in leaf-type and venation, stomatal type, wood structure, nodal structure, and gametophytes.

The small cones of *Ephedra* resemble in size and shape the so-called cones of *Cordaianthus* (Fig. 6), but the former are compound, the latter simple. This resemblance is superficial; the homology is that of the cone of *Cordaianthus* and the fertile shoot of *Ephedra*. Close similarity in basic structure exists in the cone of *Ephedra* and the cone cluster of *Cordaianthus*. The cone cluster of *Cordaianthus* and the cone of *Ephedra* (Fig. 6) are alike in gross structure: each consists of an axis bearing several to many fertile axillary branches subtended by prominent, enclosing bracts. The reproductive structures of the modern genus seem to represent a condensation and reduction form of the type found in the paleozoic genus. In *Cordaianthus*, the lateral axes are loosely arranged in a long ' raceme '; in *Ephedra*, they are compacted in a cone-like mass. The sterile appendages are many in *Cordaianthus*, two in *Ephedra*; the fertile appendages (sporophylls) are few to many in *Cordaianthus*, one (megasporophyll) or two (microsporophylls) in *Ephedra*.

In *Ephedra* fusion has accompanied telescoping and reduction, as is seen in all groups of higher plants. In most species, the two microsporophylls are adaxially connate — fused in many species to their tips, forming an axis-like structure that simulates a continuation of the shoot axis. In the ovulate shoot the two sterile appendages (bracteoles), connate marginally, are appressed to the ovule, forming the false outer integument; in the microsporangiate shoot, the bracteoles enclose the sporophylls and protect the sporangia until pollination time and are somewhat connate in a few species. The tip of the fertile-shoot axis, present in *Cordaianthus* and in the microsporangiate shoots of some primitive species of *Ephedra* (Fig. 1, *A, B*), has been lost in other species of *Ephedra*, except for a vestigial vascular supply in a few species (Figs. 2, *A*; 3, *A, c-c*).

Similarity in detail between the microsporophylls of *Cordaianthus* and *Ephedra* is remarkable. In both genera the sporophylls are flattened and bear terminal sporangia in a cluster (Fig. 4). In *Ephedra* the microsporophylls are flattened only in the primitive species where they are not connate. (The flattening of the column itself, which is at right angles to that of the sporophyll, is the result of the connation of the sporophylls side by side.) In number, the sporangia in *Cordaianthus* (as far as now known) are six to four per sporophyll; the number in *Ephedra* is four to one with the smaller numbers, clearly reduction numbers accompanying the fusion of the sporophylls. In anatomy, details of microsporophyll structure are closely alike. A single trace is present in each genus; this forks dichotomously once, twice, or three times (Figs. 4, 5) — in the upper part of the sporophyll in *Ephedra*, at the top in *Cordaianthus* — and a single branch extends into the base of each sporangium. The arrangement of sporangia within the clusters is similar (Fig. 4).

Detailed comparison of the megasporophylls cannot be complete because this sporophyll is greatly reduced in both genera. If *Cordaianthus pseudofluitans* represents a primitive type of this genus

and *C. zeilleri* an advanced type, as Florin (1951) believes and as seems probable, reduction of terminal ovules per sporophyll from two or more to one, with great shortening of the sporophyll, occurred within the genus *Cordaianthus*. Types intermediate between these two species, resembling *C. pseudofluitans* but with uniovulate, rarely forking sporophylls are known (Florin, 1950, 1951). A single terminal ovule is present also in *Ephedra* and the shortening of the sporophyll is increased greatly beyond that in *Cordaianthus*. In *C. zeilleri* the sporophyll forms a short stout stalk for the large ovule. In *Ephedra* the sporophyll is reduced to a mere base on which the ovule sits, or is lost completely. Though size is of course of no importance in considerations of phylogeny, it is noteworthy that in both *Cordaianthus* and *Ephedra* the ovule is very large in proportion to the fertile shoot and to the cone on which it is borne. Anatomical comparisons with the axis and sterile appendages of *Cordaianthus* cannot be made until the structure of these organs is known.

As between the conifers and the cordaites, the cones of *Ephedra* resemble those of the cordaites more closely than they do those of the conifers. The general
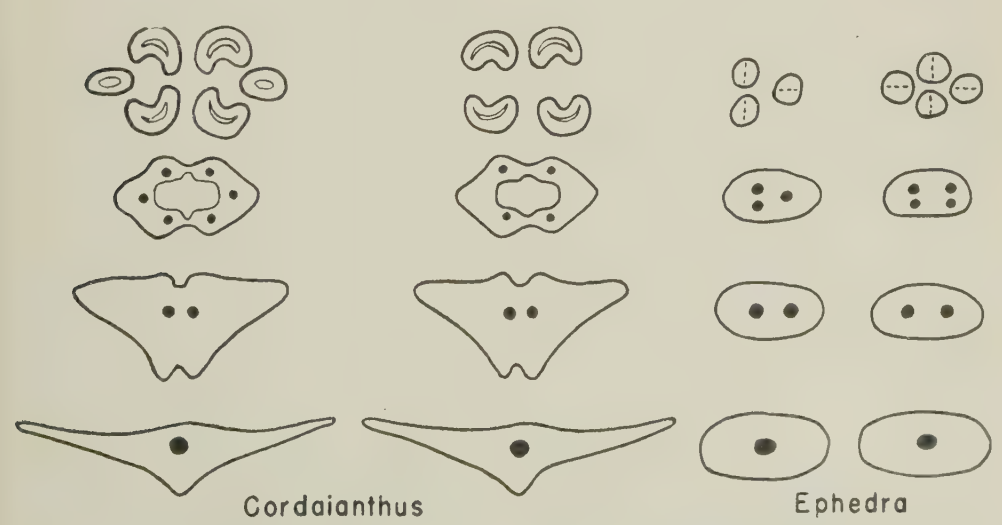

FIG. 5 — Microsporophylls of *Cordaianthus* and *Ephedra*. Cross-sectional diagrams from base to apex showing similarities in form and vascular structure and variations in anatomy related to number of sporangia. (*Cordaianthus* after Florin.)

plan of structure of the cones of the cordaites and *Ephedra* is the same. The supposedly important difference in conifer cones, of simple structure in microsporangiate cones and compound structure in ovulate cones, must now be set aside (Wilde, 1944). A new characteristic of the conifers stands out, the almost universal reduction of a cluster of male cones to one. No tendency to this reduction is seen in *Ephedra* although its *cones* — morphologically different structures — may be reduced to one. A basic, characteristic structure of the ovulate cone of confers is the axillary fertile shoot with sterile and fertile appendages fused with the axis into one structure, the " fertile scale ". There is nothing similar to this in *Ephedra*; the appendages of the fertile shoot, though reduced in number, as in the conifers, are not fused with the axis and the ovule integument as in the conifers. The microsporangia of *Ephedra* and cordaites are terminal and erect; those of the conifers are lateral and not erect. The sporophyll of the conifers has a terminal knob or blade; that of *Ephedra* and the cordaites has no such tip. The sporangium of *Ephedra* and the cordaites is supplied directly by the main vascular bundle; that of the conifers by small lateral branches of the bundle. The resemblances of *Ephedra* are clearly closer to the cordaites than to the conifers.

Evidence from reproductive structures that *Ephedra* is cordaite-like is so strong that its affinities must be with the cordaite-conifer line and not with *Gnetum* and *Welwitschia*. And this conclusion is strongly supported by evidence from the structure of the vegetative body.

Florin (1931, 1933) has demonstrated that in the gymnosperms there are two chief types of stomatal apparatus, differing in the method of development: (1) a simple type, " haplocheilic", characterized by development of the guard cells directly (by one division only) from an original epidermal cell; (2) A complex type, " syndetocheilic," in which three cell divisions occur in the formation of the guard cells from the original epidermal cell. In the simple type only two cells (the guard cells) are formed by the epidermal mother cell; in the complex type, four — sometimes six — cells are formed from the original epidermal cell. In the simple type the lateral accessory cells are sister cells of the original epidermal cell; in the complex type the lateral accessory cells are second or third generation derivatives of the original epidermal cell, sister cells of the guard-cell mother cell (or sometimes derivatives of these sister cells). Florin believes that the complex type is derived phylogenetically from the simple type; this seems evident. He states that the simple gymnosperm type differs in no way from that of the oldest land plants such as *Rhynia*. Florin shows that it characterizes universally the Pteridospermae, Cycadales, Ginkgoales, Cordaitales, Coniferales, and Ephedraceae. The complex type characterizes universally the Bennettitales, *Welwitschia*, and *Gnetum*. Evidence from broad study of stomatal structure throughout gymnosperms, living and fossil, thus supports the separation of the Ephedraceae from the Gnetaceae and Welwitschiaceae and places the family among a heterogeneous group of orders. Among these, cone and fertile-shoot morphology place it with the Coniferales and Cordaitales. It resembles these orders also in leaf type and leaf-trace plan, stelar form, and wood structure, rather than the fern-like Pteridospermae and Cycadales.

The parallel-veined linear leaf resembles that of the cordaites and conifers in general type. It usually has two traces, typically widely separated in origin, though in some species there are said to be two traces arising side by side, or there may be only one trace. In the cordaites also the leaf has one or two traces. These traces, when two, depart separately or side by side as a " double trace " ; when one, the bundle forks at once into two as does the single trace of the conifers in the primitive Araucariaceae. Little importance can be given to leaf-trace number as suggesting relationship on the basis of double traces, because such traces are common in gymnosperms. But nodal structure definitely separates *Ephedra* from *Gnetum* and *Welwitschia*. *Gnetum* has four traces (Duthie, 1912). In *Welwitschia* nodal structure is apparently as extraordinary as is the plant itself (Bower, 1881a, b). The

true leaves — two only and permanent — have a peculiar trace condition: two traces from one gap or one trace, but these are said to have, later, accessory traces added lateral to and between the original bundles, as the leaf base on the widening stem broadens through the years.

In wood structure, *Ephedra* is closer to the cordaites than to any other group. The wood is dense and the tracheides are long and slender as in the cordaites. (The density is perhaps correlated with the desert-shrub habit of the genus; similar dense wood is characteristic of many desert angiosperm genera.) In both *Ephedra* and the cordaites round-bordered pits are abundant on the radial walls, the pits arranged in one to several series. In *Ephedra* the pitting of the slender tracheides is mostly uniseriate; in the larger tracheides it is multiseriate and of the araucarian type, the pits alternating and hexangular (Thompson, 1912). In this character the pitting closely resembles that of cordaite wood. Thompson, in pointing out the close resemblance of the wood of *Ephedra* to that of the conifers, says: " All the characteristics of the tracheides of *Ephedra* are also characteristic of the conifers: the arrangement of the pits, the structure of the individual pits, the bars of Sanio, tertiary spirals, trabeculae, and resin plates. No single family of the conifers possesses all these features . . . [*Ephedra*] would seem rather to be related to some generalized ancient form." He also states that resin plates of the peculiar type found in *Ephedra* occur nowhere but in the conifers. Unfortunately all details of cordaite wood structure are not yet known, but the wood of *Ephedra* combines characters of that of the conifers and cordaites. It is of course like that of *Gnetum* and *Welwitschia* in the presence of vessels with perforations derived from round-bordered pits, not from scalariform-bordered pits as are those of angiosperms (Thompson, 1918). But the possession of vessels is recognized as evidence of high specialization in wood structure and has been attained by advanced members of several unrelated groups. Within the angiosperms it has apparently arisen independently several times from scalariform pitting. It would be strange if it had not also arisen independently

more than once from round-bordered pitting. In the light of the strong morphological differences between the three Gnetalean genera in many characters, vegetative and reproductive, it seems probable that the vessels of these three genera are further examples of parallel evolution. The possession of vessels, even of the same type, can nowhere be used as evidence of relationship.

Ephedra appears to be an isolated surviving remnant of the great cordaite stock — now becoming much better known — or of the ancestral stock of cordaites and conifers. The genus is a specialized shrub-type greatly reduced in both vegetative and reproductive structures in adaptation to arid habitats. The genus is apparently in transition from monoecism to dioecism (evidence of high specialization) with a few species definitely dioecious and other species monoecious or in a transitional state. In number, the fertile shoots of *Ephedra* are perhaps not greatly reduced from those of the cordaitean ancestor, but they are closely grouped and more completely sheltered by the large subtending bracts than in the ancestral form. The fertile axis itself is simplified, very slender and elongate in microsporangiate cones, greatly shortened in the ovulate. The elongate axis maintains the microsporangia at pollen-shedding beyond the enclosing bract and bracteoles. On the very short ovulate axis the ovule is enclosed within the bract, with the elongate micropylar apex projecting. The number of sterile appendages of the fertile axis (bracteoles) is reduced from the several or many of an ancestral form to two. The two bracteoles, more or less connate, form an enclosure for the sporophylls, usually termed the outer integument of the ovule. The bracts and bracteoles, highly modified, may serve also in seed dissemination — wing-like and papery in some species, fleshy in others.

The microsporophylls are reduced from several or many to two; the megasporophylls from several to one. The microsporangia in the more primitive species of *Ephedra* are as many or more than in *Cordaianthus*, but in advanced species are reduced in number to one per sporophyll (two on the column). The earlier forms of *Cordaianthus* (*C. pseudofluitans*) had

two or more ovules per sporophyll, but in later forms (*C. williamsoni* and *C. zeilleri*) the number is reduced to one. *Ephedra* has only one ovule and the sporophyll bearing it, already greatly shortened in *C. zeilleri*, is further reduced. Ovule number per cone is reduced in most species to two — one in the axil of each of the distal pair of bracts — and to one in the several uniovulate species where it appears (falsely) to terminate the cone axis. Similar reduction of ovules per cone to one is seen in the conifers, in species of *Podocarpus, Dacrydium, Acmopyle, Juniperus*, and probably in *Taxus* and *Torreya*; and here also the ovule appears terminal on the cone axis. In *Ephedra* the high specialization of reproductive structures parallels that of the vegetative body in indicating great age for this group.

By early definition the Gnetales were set apart from other gymnosperms chiefly by the compound nature of both ovulate and microsporangiate cones and by the presence of vessels, long micropylar tubes, and supernumerary integuments.

In the conifers, only the ovulate cones are compound. In the cordaites, the same condition was supposed to obtain, but this interpretation has been shown to be in error by the studies of Schoute (1925) and Florin (1939), and both cone types in the cordaites are now recognized as simple. The lack of uniformity in gross structure of the ovulate and microsporangiate cones of the conifers is now explained by the interpretation of the simple solitary microsporangiate cone of most conifers as a solitary surviving unit of an ancient microsporangiate cone cluster (Wilde, 1944), which still survives in *Podocarpus* (section *Stachycarpus*) and probably also in *Taxodium* and *Cunninghamia*. In the conifers the simple microsporangiate cone is the homologue of one of the fertile short shoots (" fertile scales ") of the ovulate cone.

The racemose cluster of cones of *Cordaianthus* is morphologically the equivalent of the microsporangiate and ovulate cones of *Ephedra* and of the ovulate cone of the Pinaceae and most other conifers (Fig. 6). It is homologous also with the racemose clusters of microsporangiate cones of *Podocarpus* (section *Stachycarpus*), with branches of the paniculate cluster of

Taxodium, and with the axillary clusters of similar cones in *Cunninghamia*. One unit of the cordaite cluster is homologous with the fertile shoot of *Ephedra* and with the solitary microsporangiate cone of most conifers.

The clusters of cones (" inflorescences ", " dichasia ") of *Ephedra* have no known equivalent in the Cordaitales or in living Coniferales [with the possible exception of the recently described *Araucaria bernieri* Buchholz (Buchholz, 1949) from New Caledonia, where 2-7 large ovulate cones occur in a cluster]. Whether these clusters are borne on a single stem, or consist of solitary cones grouped about a main axis as in *A. angustifolia* and some species of *Pinus* is uncertain.

With the new understanding of the nature of the cones of conifers and cordaites, the Ephedrales are no longer set off from the Cordaitales and Coniferales by the possession of compound structure in both ovulate and microsporangiate cones. Basic gross structure is the same in all three groups. The conifer line stands apart from the other two groups by extreme simplification of the microsporangiate cone cluster, reduction to one unit. The cordaites appear superficially very different from the Coniferales and Ephedrales because their cone clusters have not been condensed into compound cones as in the ovulate cone of conifers and in both ovulate and microsporangiate cones of *Ephedra*. In living conifers similar lack of condensation survives in *Podocarpus*, which is in some characters the most primitive living conifer genus.

Individual cones of *Ephedra* with both ovulate and microsporangiate fertile shoots (an occasional feature in many species and one perhaps characteristic of *E. campylopoda*) have been referred to occasionally as " bisexual cones " and " bisexual inflorescences ", partly in proof of similarity of *Ephedra* to *Welwitschia* and *Gnetum*. This, as " evidence of relationship ", has been mentioned earlier in this paper as an error, since the structures compared are not the same (*cones* in *Ephedra*, *fertile shoots* in the other genera). The same error underlies in large part the likening of such cones of *Ephedra* (with ovules in the distal axils and microsporophylls below) to the

angiosperm flower. Here a cone (a compound cluster of fertile axes) is likened to a flower (a single, simple fertile axis). Fertile axes are not homologous with appendages. In these " bisexual cones " even the position of microsporangiate and ovulate fertile axes is not constant. The microsporangiate, as well as the ovulate, may be at the top; the two types may be mixed in position; or even one of the members of the distal pair of fertile shoots may be ovulate, the other microsporangiate. The distribution of the two types of fertile shoots in cones of *Ephedra* would not therefore be significant in the origin of the angiosperm flower, even if *Ephedra* seemed to fall in a possible ancestral line of the angiosperms. The *fertile shoots* of *Welwitschia* and *Gnetum* are of course bisporangiate in contrast with the rigidly monosporangiate shoots of *Ephedra*. (It is doubtful that the fertile shoot of *Gnetum* is truly bisexual.)

In general structure of ovule and female gametophyte *Ephedra* resembles the conifers. But *Ephedra* has a well-defined pollen chamber and its archegonia are deeply sunken in the gametophyte. Deeply sunken archegonia are present in some primitive conifers (Araucariaceae and Podocarpaceae) and a pollen chamber is characteristic of primitive gymnosperms. The pollen chamber of *Ephedra* has been considered by some authors as secondarily formed, not primitive. Such a theory is of course necessary to support the view that the angiosperms have been derived from the conifers through the Gnetales because there are no pollen chambers in the conifers. Comparison of ovule structure in *Ephedra* and the cordaites cannot be made because little is known in detail of ovule and seed structure in the cordaites.

Relationship of Ephedra with Gnetum and Welwitschia

Florin (1931) has pointed out that, in discussions of relationship between the Gnetales and other gymnosperms, resemblances have been seen, on the one hand, between the more advanced genera, *Welwitschia* and *Gnetum*, and the Bennettitales; on the other hand, between the

Cordaianthus   Ephedra   Podocarpus   Pinus

FIG. 6 — Diagrams showing homologous structures: cone cluster of *Cordaianthus* (shown broken to indicate that cluster may be either microsporangiate or ovulate); cone of *Ephedra*; cone clusters of *Podocarpus* (section *Stachycarpus*), ovulate and staminate; ovulate cone of *Pinus*.

genus commonly recognized as primitive, *Ephedra*, and the conifers. For the position of *Ephedra*, this study supports the resemblances with strong evidence, although indicating that this genus is closer to the cordaites than to the conifers.

Some additional resemblances and differences between the three Gnetalean genera merit consideration. The long micropylar tube, considered a character that holds these genera together, occurs elsewhere among gymnosperms only in the Bennettitales. In the Bennettitales its form is apparently related to pollination, the tube-like tips of the ovules projecting between the swollen paraphysial tips of the sterile sporophylls which enclose and protect the ovules. Similarly in the Gnetales, bracts and bracteoles enclose the ovules at pollination time and the tubes, like the tips of the microsporophylls, project beyond them. The presence of the long tube in all three genera may be explained by parallel development, for *Welwitschia* and *Gnetum* perhaps by inheritance from Bennettitalean stock from which these genera show evidence of derivation.

The presence of supernumerary integuments as an indication of close relationship among the three genera should be disregarded. As Lotsy (1899) stated long ago, there is only one " true "[2] integument in all three genera. The outer " integuments " are pairs of connate bracts that simulate integuments. This is evident in *Ephedra* and *Welwitschia* by even superficial study, and anatomy supports the interpretation. The two outer integuments in *Gnetum* are apparently also pairs of bracts similar to those at nodes lower on the peduncle.

Evidence from the gametophytes of the three genera is prominent in all discussions of relationship among them. The evidence for non-relationship of *Ephedra* to the other genera consists chiefly of the presence of (1) a typical female gymnosperm gametophyte whereas the other genera have a gametophyte unlike that of any other group; (2) a vestigial tent-pole at the apex of this gametophyte (a structure unknown in the cycadophyte line);

2. The nature of the integument throughout seed plants — telomes, leaf-segments, bracts, a structure *sui generis* — is still uncertain.

(3) a male gametophyte with two prothallial cells and stalk nucleus in *Ephedra*, structures absent in the other groups. Fertilization and early embryogeny in *Ephedra* differ little from that in the conifers, but in the other genera they are neither gymnosperm-like nor angiosperm-like. The structure of the gametophytes and embryo strongly support the separation of *Ephedra* from *Welwitschia* and *Gnetum* and emphasize its conifer-like and cordaite-like nature. So also does the vascular anatomy of the ovule. In *Gnetum* and *Welwitschia* a vascular supply passes half-way up the true integument ; in *Ephedra* it ends in the base of the ovule (Thoday & Berridge, 1912 ; Pearson, 1929).

Ephedra differs greatly from *Gnetum* and *Welwitschia* in primary structure of the stem. Difference in leaf-trace number has already been noted. Differences in stelar structure have been pointed out by Thompson (1912) and Pearson (1929). The primary stelar structure of the stem of *Ephedra* is simple with a small number of vascular bundles arranged according to a simple pattern which suggests that of conifers, whereas in the other genera there is a much larger number of bundles which form a complex mass.

The stem apex of the Gnetales has not been studied critically in the light of present-day views except in *Gnetum* (Johnson, 1950). (That of *Welwitschia* is lost after the seedling stage.) Pearson (1929) described the stem apex of *Ephedra* as massive in type, with the limits of plerome, periblem, and often even of dermatogen ill-defined. Gifford (1943) calls it highly specialized. As described by both these authors it differs greatly from that of *Gnetum* which is angiosperm-like in the possession of a tunica and corpus. (Johnson notes that the stem apex of *Gnetum* resembles that of cycads, perhaps a significant remark in the consideration of a Bennettitalean origin for *Gnetum*.) Both the primary structure of the stele and the structure of the apex of the stem support the wide phylogenetic separation of *Ephedra* from the other genera.

Opposite leaves, binary whorls of sporophylls, dicotyledonous embryos, and dichasial inflorescences have been commonly

cited as less important distinguishing characters of the Gnetales. These characters are, however, all unimportant. Dicotyledonous embryos are not significant as evidence of relationship; they occur in nearly all major groups of seed plants. Decussate phyllotaxy is high expression in leaf arrangement, and sporophyll arrangement commonly follows leaf arrangement, as in the highest conifers.

Similarly, dichasial inflorescences, with reduction to a single unit, occur in other groups. In *Ephedra* the reduction of a complex dichasial cluster of cones to a solitary cone by loss of lateral branches and telescoping of lower internodes is further evidence of high specialization in the genus. (This simplification is similar to that characteristic of most conifers where the male cones are reduced from many to one, though the cones of the two groups are not homologous and the cluster types are different.)

Coulter and Chamberlain (1910) are inconsistent in their statements as to relationships of the Gnetales. They state (p. 404) : " It is recognized that in the evolution of strobili among gymnosperms there were probably two distinct tendencies: a monosporangiate strobilus (Cycadales, Cordaitales, Ginkgoales, Coniferales); and a bisporangiate strobilus with the anthostrobilus arrangement of sporophylls (Bennettitales, Gnetales) and leading to angiosperms." Here *Ephedra*, apparently considered bisporangiate like the other genera of the Gnetales, is set wholly apart from the cordaites and conifers. Yet Coulter and Chamberlain say (p. 402) : " It is clear that *Ephedra* is more closely related to other gymnosperms than *Welwitschia* and *Gnetum*; that if it is to be connected with them at all, it is most reasonably connected with the Coniferales . . . whatever may be the connection of *Ephedra* with other gymnosperms it cannot be separated from *Welwitschia* and *Gnetum*."

Sykes (1911), after detailed study of *Welwitschia*, stated that it is " probable that *Welwitschia* is not closely allied to the other Gnetales. It most closely resembles the cycads and the Medulloseae ". Thoday (Sykes) also says, in editorial comment in Pearson (1929), that " it is

not impossible . . . that *Gnetum* and *Welwitschia* do not belong to the same line of descent as *Ephedra* ".

There were several early suggestions that the Gnetales might be related to the Cordaitales. Saporta and Marion (1885) considered the Gnetales cordaite-like because the microsporangia are erect and terminal on appendages and Thibout (1896) likened the stamens of *Ephedra* to those of the cordaites. Solms-Laubach (1887) believed that the ovulate flowers of the cordaites should be compared with those of *Ephedra*. Bertrand (1911) and Thibout (1896) also considered this relationship probable. Scott (1909) compared the Gnetales with the cordaites and Bennettitales but concluded that the affinities of the Gnetales are with the Mesozoic Bennettitales. The view that *Ephedra* is related to the cordaites is by no means new.

The circinate vernation of the column in *E. fragilis* has been considered evidence of cycadean or Bennettitalean origin, but this vernation, as illustrated by Thoday and Berridge (1912), is not typical circinate vernation. The column tip is merely reflexed, closely like the reflexing of young leaves in *Botrychium*, which is considered as non-circinate in contrast with the true circination of leptosporangiate ferns. No other species of *Ephedra* has been reported to show this reflexing, which doubtless is related to enclosure in early stages by the bracteoles and is therefore without phylogenetic significance.

That *Ephedra* is not in any way related phylogenetically to the other genera of the Gnetales seems certain. The view that it belongs to the general cordaite-conifer line appears well founded, with derivation from either the cordaite line or the ancestral stock of that line.

As pointed out by Markgraf (1926), all three genera appear to be survivors of ancient gymnosperm stocks, because of their wide and scattered distribution over the earth and because they show characters of the " Urgymnospermae ", yet are very highly specialized in habit. In the words of Lignier and Tison (1912) they form "un ensemble d'une ancienété indiscutable ". The few surviving members of these stocks have attained a level of high specialization

much like that of some angiosperms. Though *Ephedra* in many characters is more primitive than the other genera, it is, like them, highly specialized. Each genus undoubtedly represents the terminal form of a long line of specialization, the three lines independent of one another, and *Ephedra* not even distantly related to the other genera.

The Bearing of Ovule Position in Ephedra on Sahni's Classification of Gymnosperms

Schoute (1925), after demonstrating that the ovule of the cordaites is appendicular, urged that Sahni's (1921) division of the gymnosperms into Phyllospermes and Stachyospermes be abandoned. He says (p. 125) that in his demonstration of the true position of the ovule in the cordaites " Sahni perd son dernier support ". He mentions the Gnetales only later on the page, and it is apparent that he believed the ovule in this group to be foliar. He says: " C'est seulement dans une partie des Taxacées, dans le *Ginkgo* et dans les Gnetacées que le sporophylle est tellement reduit qu'il n'en reste souvent que l'ovule lui-même." He states further: " Il me semble que les Gymnospermes comme toutes les Angiospermes et toutes les Ptéridophytes, sauf les Psilophytines, ne forment leurs sporanges que sur des sporophylles."

Scott (1923) in his " Studies in Fossil Botany " opposed Sahni's division of gymnosperms into Phyllospermes and Stachyospermes on the ground that there was insufficient knowledge of the morphology of the ovulate cones of conifers and cordaites. But in the years since Scott's statement, Florin (1950) has reinvestigated the cones of the cordaites and concurs in Schoute's interpretation of their structure. He has also studied the evo-

lutionary history of the conifers with thoroughness (1938-1945). Others have contributed much to the knowledge of the conifer cones, especially of their anatomy. The bases for judgment of Sahni's classification lacking to Scott have been provided in the intervening years and give support to Schoute's opinion.

Schoute, at least in his own mind, had already removed the Gnetales from the Stachyospermes. The present paper brings together the evidence for the removal of *Ephedra* from that group and supports Schoute's position. Though Lam (1948, 1950) has recently been extending Sahni's division of seed plants into the angiosperms, the basis of Lam's treatment is without morphological foundation because it disregards completely the evidence of thorough morphological comparisons, and especially that of anatomy (Eames, 1951). The writer concurs in Schoute's opinion that the division of gymnosperms into phyllosperms and stachyosperms is not justified.

Summary

The recent taxonomic break-up of the old order Gnetales into three orders — Ephedrales, Welwitschiales, and (a retricted) Gnetales — is strongly supported by morphological evidence, especially that of anatomy. Phylogenetically the Ephedrales stand far from the other orders and are not even distantly related to them. The ovule in *Ephedra* is appendicular and not cauline, as commonly interpreted. Resemblances are close to the cordaites, where the ovule has also recently been shown to be appendicular. *Ephedra* is apparently a derivative of ancient cordaite-conifer stock. The appendicular position of the ovule supports Schoute's view that Sahni's classification of gymnosperms is not valid.

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REVIEWS

MACDONALD, J. A. 1951. "Introduction to Mycology." Pp. 177. Butterworths Scientific Publications. 15s.

THIS excellent little book of 177 pages is divided into 21 chapters followed by an index. The first three chapters are devoted to general considerations about fungi and include the principles that govern their classification. The next chapter deals with Myxomycetes; five chapters each deal with Phycomycetes and Ascomycetes respectively and four with Basidiomycetes. The Fungi Imperfecti, Mycorrhiza and Lichens are assigned only one chapter each. From a wealth of interesting material, the author has wisely drawn upon the more important facts to illustrate the major phenomena about fungi. These are illustrated by reference to a limited number of examples of " types " whose life-cycles in the various stages of their growth unfold a range of knowledge that is so necessary to those who desire to make a more advanced study of fungi.

The accounts of the orders and the families are rather fragmentary but those of the types are clear and satisfactory. The chapters on the relationships of Phycomycetes, Ascomycetes and Basidiomycetes are briefly but clearly written; one would have liked to see fuller accounts of the phenomena of heterothallism and of sex in Ascomycetes and Basidiomycetes, especially the rusts. The author has not used the term " diploidization " any where and " brachymeiosis " is mentioned but not defined.

The book has been excellently produced but the figures are disappointing, and most of them are rather poor. It is hoped that in a subsequent edition an attempt would be made to improve them. The author refers to the zygote of *Synchytrium endobioticum* (which is an Oomycete) as a zygospore and on p. 79 *Taphrina*

deformans is spelt *Taphrina deformers*. Gasteromycetes, which form a sub-class of Basidiomycetes, are treated as an order. No doubt these few errors and omissions will be rectified in the next edition. The book will be found quite useful by those for whom it is meant and the author is to be congratulated on a nice job well done.

B. B. MUNDKUR

LILLY, V. G. & BARNETT, H. L. 1951. " Physiology of the Fungi." Pp. 464. McGraw-Hill Book Co. Inc. $7.50.

IN recent years a number of treatises on the Physiology of the Fungi have appeared in quick succession, some exhaustive, others elementary. This bears testimony to the increasing interest building up around this group of micro-organisms, particularly after the discovery of industrially important fermentations by many fungi, the classical discoveries of antibiotic substances and the renewed efforts in many laboratories to understand the intricacies of the metabolic processes of these micro-organisms. The book under review is well and authoritatively written, particularly when the authors deal with "Hydrogen-ion concentration", "Vitamins and Growth factors " and " Fungi as Test Organisms ", in which branches their main contributions lie. Indeed, the chapter on " Vitamins and Growth factors " is stimulating to the research worker who normally does not get in one book all the references on this subject. The distinction between vitamins and vitamers and the capacity of various fungi to synthesize their vitamins from pure chemicals in a synthetic substrate are admirably dealt with. Among the eighteen interesting chapters only a few need special mention here: " Enzymes and their action ", " Essential metallic elements ", " Factors influencing sporulation of fungi ", and "Physiology of parasitism and resistance".

The last unnumbered chapter on " Laboratory Exercises " is a useful termination from where the post-graduate student can have material for practical initiation into fungal physiology. There is no doubt that the authors have brought together a mass of data on varied problems connected with this vital subject in a simple and critical manner, the text itself being profusely illustrated with figures and photographs. It may, however, be mentioned that there is a certain amount of what might be called ' lack of finesse ' in interpreting results, and in such situations the authors have merely been content with stating facts and do not offer any critical overall comments on intricate physiological problems. It is true that in a rapidly growing multi-faceted experimental science like this much has to be left to the readers' imagination for interpreting data. Be that as it may, there is no doubt that the sum total effect is a logical sequential story of the Physiology of Fungi which is in keeping with the high traditions of the publishers.

A fairly large number of misprints and a few incorrect cross references have crept into the text. This is regrettable. The mode of citation of journals is not strictly in accordance with International convention. Despite these minor blemishes the book is to be warmly commended to all students and research workers interested in the Fungi and their Physiology.

T. S. SADASIVAN

WESTCOTT, C. 1950. " The Plant Doctor" (3rd Revised Edition). Pp. 231. J. B. Lippincott Co., Philadelphia and New York. $3.00.

THE popular treatment of an important subject like plant protection, including both fungicides and insecticides, is a welcome sign in these days when plant quarantine and plant protection are very much in the air. This little book represents years of experience the author has had in administering proper recipes of the innumerable plant protection chemicals that have flooded the market. It is

written in very clear style especially for the use of the growers of the New World where the science of plant disease diagnosis and treatment with a bewildering galaxy of chemical products has perhaps reached its zenith.

The book is divided into chapter heads for each month of the year beginning with February and ending with November. Under each month the diseases are listed in chronological order with effective line drawings indicating the pests and the damaged plants. The remedial measures are discussed straightaway. There are five chapters for " Special troubles " in the middle west, southeast, southwest and northwest of the United States of America and in California where the bulk of the finest orchards are situated. Over 62 pages are devoted to " Alphabetical Miscellany " which includes short notes, more like a ready reckoner, on hosts, vectors, pests, fungi, bacteria, common names of diseases, tools, sprayers, insecticides, fungicides, etc.

Those interested in plant protection would find this book useful. To those who have already worked on these lines in India the book would be a reminder of what this country expects of them.

T. S. SADASIVAN

NELSON, A. 1951. " Medical Botany." Pp. 544. E. & S. Livingstone, Edinburgh. 30s.

THE author is already well known on account of a previous book entitled " Principles of Agricultural Botany ". The present work deals with botany in relation to human and veterinary medicine.

Much interesting information is provided on the composition of the foods, drugs and poisons obtained from vegetable sources. The first section gives a general account of plant foods in relation to quality. The second and largest section deals separately with cereals, pulses, oil seeds, stem, leaf and root vegetables; and foodstuffs of cryptogamic origin. The third section deals with drugs, poisons and stimulants; and the last with plants as causal agents of disease.

The information is all very readably presented and the reviewer derived much profit from it. In his opinion not only students going for the medical college but even others taking a general course in botany should read the book.

The illustrations are of good quality. On p. 473 *Lycopodium* is mentioned under mosses while *Sphagnum* which has frequently been used in surgical dressings has not been mentioned at all.

P. MAHESHWARI

BISSET, K. A. 1950. " The Cytology and Life history of Bacteria." E. & S. Livingstone Ltd., Edinburgh. 18s. 6d.

OWING to its minuteness little is known of the internal structure of the bacterial cell. This, as the author points out in his " Introduction ", is no doubt due to the fact that the distorted vestiges of the cell which survive the customary technique of drying and heat-fixation followed by Gram's stain are not suited to the task of an elucidation of nuclear structure. For this the author recommends the avoidance of heat and the use of the Feulgen reaction.

When studied with the help of proper methods many bacteria are found to be multicellular with numerous cross-septa derived from the cell wall, the flagellae are " spiral in form and composed of protein ", a nuclear membrane can be distinguished at certain stages of the life cycle, and " most frequently the nucleus is in the form of paired chromosomes " which are short rods lying *transversely* to the long axis of the cell. It is further stated that in some forms at least the vegetative form is haploid, and a sexual fusion is immediately followed by reduction (except in *Streptomyces*) and the formation of haploid spores. The presence of sexuality is confirmed on genetical evidence, and the data and arguments presented are of a most stimulating type. The printing and general get up of the book are excellent.

P. MAHESHWARI

SKOOG, FOLKE (Editor). 1951. " Plant Growth Substances." Pp. 476. University of Wisconsin Press. $6.00

ON account of the large amount of work done all over the world on hormones and in order to gain a proper perspective of the present status of this field, a committee of several workers organized, in 1949, a symposium in which besides the usual formal lectures there were many group discussions. Prof. Folke Skoog served as the editor and funds were made available by the Wisconsin Alumni Research Foundation.

No less than 40 persons have contributed to the volume. Of special interest to the general reader are Haagen-Smit's and F. W. Went's historical summaries. A. F. Blakeslee has written an interesting article on the " Control of evolution and life processes in plants " in which he has discussed the ways of removing the various barriers to crossability in plants. P. W. Zimmermann has dealt with the many ways in which hormones are employed in modern agricultural and horticultural practices. An article of special interest to the plant morphologist is the one entitled " Histological responses to growth-regulating substances " in which J. M. Beal starts with the classical work of Kraus, Brown and Hamner, published in 1936, and shows how, in the presence of growth-regulating substances and proper food and nutrient supply, cells of recognizably differentiated tissue systems may dedifferentiate, become embryonic, and proliferate. From these derivatives, distinct types of tissues may be differentiated resulting in the healing of wounds, rooting of cuttings and delay of abscission. Immediately after Beal, B. E. Struckmeyer discusses the comparative effects of growth substances on stem anatomy paying special attention to the questions as to why do most dicotyledons but only a few monocotyledons respond to 2, 4-D; what is the possible rôle of α-naphthaleneacetic acid in delaying calcium deficiency symptoms; and how does α-naphthaleneacetic acid function both in the thinning of apples as well as preventing their pre-harvest drop?

Of interest is also the paper by J. van Overbeek on the " Use of growth substances in tropical agriculture ". The author emphasizes that in the zeal for quick results applied research must not be allowed to starve out fundamental research on which alone all progress ultimately depends.

The section on growth substances in vegetative development commences with a very valuable article by P. R. White dealing with some aspects of tissue culture, while to those devoted to experimental embryology the paper by Nancy Kent Ziebur will prove most suggestive. She attempts a discussion of two major problems: (1) how is the embryo fed, what is the nature of the food material it receives and from which of the surrounding tissues is it obtained; and (2) to what extent is the pattern of embryonic growth autonomous.

A mixed lot of 7 articles is collected in the section dealing with " Growth substances in reproductive development ", all written by distinguished persons: Raper, Smith, Murneek, Roberts, Gustafson, Muir and Wittwer. Murneek emphasizes the importance of hormones in the endosperm-embryo relationship and Roberts reports the isolation of a flowering hormone from plant extracts.

One section is devoted to the rôle of hormones in the physiology of tumors and other overgrowths — a subject which has aroused much interest in recent years. The last section concerns vitamins and amino acids.

All told the book is a most useful addition to a field of research which embraces many branches of botany. The only criticism the reviewer has to make is that there is much overlapping of the material resulting in a conglomeration without co-ordination, and there is no index. This impairs the utility of the volume to the general reader.

P. MAHESHWARI